Toxicon:X 6 (2020) 100039

Contents lists available at ScienceDirect

Toxicon: X

journal homepage: www.journals.elsevier.com/toxicon-x

Toxinology provides multidirectional and multidimensional opportunities: A personal perspective

R. Manjunatha Kini *

Protein Science Laboratory, Department of Biological Sciences, Faculty of Science and Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

ARTICLE INFO

Keywords:
Accelerated evolution
Tissue-selective expression
Gene duplication and neofunctionalization
Regulation of toxin expression
Targeted drug delivery

ABSTRACT

In nature, toxins have evolved as weapons to capture and subdue the prey or to counter predators or competitors. When they are inadvertently injected into humans, they cause symptoms ranging from mild discomfort to debilitation and death. Toxinology is the science of studying venoms and toxins that are produced by a wide variety of organisms. In the past, the structure, function and mechanisms of most abundant and/or most toxic components were characterized to understand and to develop strategies to neutralize their toxicity. With recent technical advances, we are able to evaluate and determine the toxin profiles using transcriptomes of venom glands and proteomes of tiny amounts of venom. Enormous amounts of data from these studies have opened tremendous opportunities in many directions of basic and applied research. The lower costs for profiling venoms will further fuel the expansion of toxin database, which in turn will provide greater exciting and bright opportunities in toxin research.

1. Introduction

Toxins are substances produced by living organisms that interfere in the physiological processes of the victim, which could be a prey, predator or a bystander. Venoms are complex mixtures of tens or hundreds of toxins and are produced in specialized organ. In venomous organisms, the toxins are actively administered into the victim by a bite or sting. In contrast, toxins produced in poisonous organisms enter the victim passively when the victim takes bite of the organism. In both situations, the victim suffers from mild to severe disturbances in various physiological processes leading to debilitation and death depending on the toxin dose. Toxins have evolved as the part of either the defensive strategy against predators or the offensive strategy to capture and subdue the prey. In poisonous organisms, as they do not actively 'hunt', toxins are produced as a deterrent against predators. Because of the importance of toxins for their survival, venomous/poisonous organisms have evolved multiple times throughout the phylogenetic tree resulting in myriads of toxins with varied structures, functions and targets in distinct physiological systems (Casewell et al., 2013). Toxinology is a multidisciplinary science, which aims in understanding various aspects of toxins including, but not limited to, the structure, function, mechanism, origin and evolution, clinical aspects and strategies to resolve the toxicity. In this review, although I focus only on toxins from venomous animals, most of these concepts fit toxins from other organisms.

Although a number of animal venoms cause mild to severe discomfort, the bites and stings from only a small number of venomous animals, such as snakes and scorpions, resulted in death and debilitation of humans (Chippaux and Goyffon, 2008; Abroug et al., 2020; Gutiérrez et al., 2017; Bawaskar and Bawaskar, 2019; Hunter et al., 2019; Waiddyanatha et al., 2019; Williams et al., 2019). Thus, most of the initial studies focused on the isolation and characterization of individual highly toxic components of these venoms and on the strategies to neutralize the pathological symptoms caused by the toxins. With the increased sophistication and high resolution in purification and characterization methods, it was possible to evaluate the toxins that could be isolated in smaller amounts. With the advent of Omics technologies, it has become much easier to complete deep transcriptomes from minimal amount of tissues, proteomics with miniscule amount of crude venoms, genomes with tiny amounts of tissues and combinations thereof. With these new technologies, we can obtain toxin profiles of venoms and complete the identification and classification all components, taken together as venomics (Lomonte et al., 2008; Calvete, 2013; Tasoulis and Isbister, 2017; Modahl et al., 2020). This brings us to crossroads and the question 'Where do we go from here?'. Here, I will describe my personal

* Protein Science Laboratory Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore, 117543, Singapore.
E-mail address: dbskinim@nus.edu.sg.

https://doi.org/10.1016/j.toxcx.2020.100039
Received 13 April 2020; Received in revised form 28 April 2020; Accepted 5 May 2020
Available online 11 May 2020

perspective on multidirectional and multidimensional opportunities and fascinating future in the field of toxinology (Fig. 1).

2. Antivenom research

Conventional antivenoms comprise antibodies or antibody fragments derived from the plasma of larger mammals (typically horses) that have been immunized with venom(s) (Lalloo and Theakston, 2003; Theakston et al., 2003; Gutiérrez et al., 2011). Quick and timely treatment of the victims through intravenous administration of antivenoms is the only accepted treatment of the severe medical emergency and concomitant multiple organ failure due to envenoming. Antivenomics helps to evaluate the quality and neutralizability of antivenoms and identifies toxins that exhibit poor antigenicity/neutralizability (Lomonte et al., 2008; Calvete et al., 2018). These studies indicate that despite best efforts, typical antivenoms contain only 5–46% toxin-binding antibodies (Rawat et al., 1994; Segura et al., 2013; Herrera et al., 2014; Sanz et., 2018). These data along with others supporting the inherent drawbacks of antivenom use (Kini et al., 2018) provides impetus for the development of next generation strategies to replace more than a century old technology (Calmette, 1894a, 1894b; Calmette, 1896). Since the first Food and Drug Administration (FDA)-approved therapeutic monoclonal antibody (mAb) OKT3 (muromonab) in 1986 (Hooks et al., 1991), particularly human therapeutic mAbs and their fragments, have become the mainstay in the development of biologics for acute and chronic treatments of various human diseases (Walsh, 2014, 2018). The works of George P. Smith and Gregory P. Winter "for the phage display of peptides and antibodies" (Parmley and Smith, 1988; McCafferty et al., 1990) together with that of Frances H. Arnold "for the directed evolution of enzymes" (Chen and Arnold, 1991, 1993; Arnold, 2019) laid the foundation for the development of therapeutic antibodies. All three shared the 2018 Nobel Prize in Chemistry. Phage display for panning and selection combined with directed evolution as a reliable optimization algorithm have made selection and maturation of therapeutic antibodies somewhat routine (Hoogenboom et al., 1998; Rodi and Makowski, 1999; Sidhu, 2000). Recent improvements in antibody-expression yields and manufacturing processes have made mAbs as a cost-effective option (US$ 20–80/g) for the treatment of various diseases (Klutz et al., 2016; Pollock et al., 2017; Yang et al., 2019). Thus, there has been a dramatic rise in the use of therapeutic mAbs, with 78 mAbs approved by the FDA or the European Medicines Agency (EMA) and the annual global mAb market reached US$100 billion in 2017 (Hooft van Huijsduijnen et al., 2020). In 2018, 11 more mAbs were approved by the FDA (Mullard, 2019; Baedeker et al., 2019) and over 570 mAbs are in clinical development and this number of mAbs approved and in clinical development are growing in 2020 (Kaplon and Reichert, 2019; Kaplon et al., 2020). With these considerations, we proposed the production of Biosynthetic Oligoclonal Antivenom (BOA) based on recombinantly expressed oligoclonal mixtures of human mAbs or their fragments for snakebite treatment (Kini et al., 2018). Current technologies for selection, production, and characterization, will allow the development of next generation of antivenoms against snake venom toxins (Laustsen et al., 2018; Laustsen and Dorrestijn, 2018; Ledsgaard et al., 2018; Roncolato et al., 2015). The ability to complete high quality genomes of venomous snakes (Vonk et al., 2013; Yin et al., 2016; Shibata et al., 2018; Perry et al., 2018; Schield et al., 2019; Suryamohan et al., 2020) and to produce toxins through organoids (Post et al., 2020) will open avenues for BOA. Human therapeutic antibodies provide several potential advantages over conventional horse-derived antivenoms. They include compatibility with human victims, enrichment of toxin-neutralizing antibodies, consistent and reproducible quality, tailor-made mAbs and their fragments with optimal pharmacokinetics and pharmacodynamics, better safety and cross reactivity with related toxins (For details, see Kini et al., 2018). BOA can possibly be combined with repurposed small molecule, synthetic enzyme inhibitors (Bulfone et al., 2018; Bryan-Quirós et al., 2019; Lewin et al., 2018a, 2018b; Salvador et al., 2019; Albulescu et al., 2019). Thus, venomics helps expanding our abilities to develop next generation antivenoms.

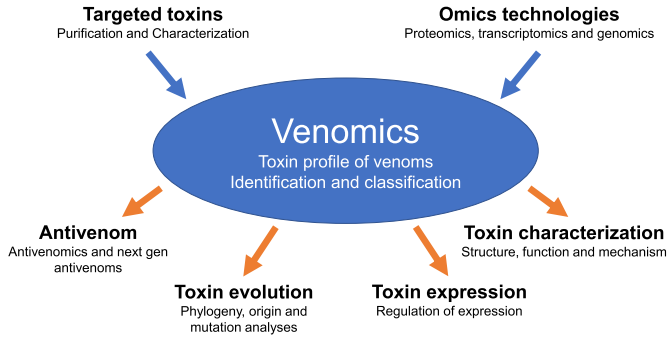

Fig. 1. An overview of multidirectional and multidimensional opportunities in toxinology.

3. Toxin evolution

Venoms have evolved as chemical means for prey capture and competitor and/or predator deterrence (Fry et al., 2009). In the complexity of a food web, these indirect interactions control entire ecosystems. The sequence information obtained through proteomics, transcriptomics and genomics, in addition to providing the toxin profiles of venoms, opens new avenues in understanding the evolutionary history of toxins and in resolving these predator-prey interactions. Most accepted hypothesis for the evolutionary origins of toxin families suggests the duplication of nontoxic cognate genes expressed in other tissues, and subsequent change in tissue-specific expression (leading to venom gland expression) and neofunctionalization through modification of the structure and function. This hypothesis was supported by the structural similarity between various toxins with nontoxic proteins. With the initial protein sequence information, that evolution of protease inhibitors and ribonuclease (Strydom, 1973a,b) and phospholipase A_2 (PLA2) (Halpert and Eaker, 1975) as snake venom toxins were proposed. Subsequent sequence studies have indicated that elapid and viperid PLA2s originated from pancreatic and synovial (platelet) PLA2 lineages, respectively (Heinrikson et al., 1977; Seilhamer et al., 1989). With more sequence information of the toxins and their related proteins, it became clear that several other snake venom toxins originated from cognate genes encoding proteins that played roles in normal physiological functions. These included sarafotoxins/endothelins (Kochva et al., 1982, 1993; Weiser et al., 1984; Wollberg et al., 1988; Yanagisawa et al., 1988), cobra venom factor (Vogel et al., 1984), natriuretic peptides (Schweitz et al., 1992), nerve growth factor (Cohen and Levi-Montalcini, 1956; Inoue et al., 1991; Kostiza and Meier, 1996), vascular endothelial growth factor (Komori and Sugihara, 1990; Komori et al., 1999; Yamazaki et al., 2003), snake venom metalloproteases (Takeya et al., 1990; Hite et al., 1992, 1994; Au et al., 1993; Paine et al., 1992, 1994; Bjarnason and Fox, 1995; Jia et al., 1996), neurotoxin/Ly-6/lynx1 (Fuse et al., 1990; Fleming et al., 1993; Chang et al., 1997; Lyukmanova et al., 2011; Miwa et al., 1999) and acetylcholinesterase (Cousin et al., 1998). Based on the protein sequences, we showed that venom prothrombin activators are structural and functional homologues of blood coagulation factors; group D prothrombin activators are similar to factor Xa (Joseph et al., 1999; Rao et al., 2003a), while group C prothrombin activators are similar to factor Xa-factor Va complex (Rao and Kini, 2002; Rao et al., 2003b). Some of the toxins exhibited significant diversification, while others showed minor structural and functional changes. Thus, toxins were thought to be evolved from endogenous genes regulating in normal cellular pathways (Ohno et al., 1998; Fry, 2005; Fry and Wüster, 2004; Kini and Chan, 1999). Recent genomes of venomous snakes (Vonk et al., 2013; Yin et al., 2016; Shibata et al., 2018; Perry et al., 2018; Schield et al., 2019; Suryamohan et al., 2020)

have confirmed many of these assertions. Phylogenetic analyses of toxin families also indicate the possibility of reverse recruitment of toxin genes back to physiological roles (Casewell et al., 2012). With increasing volumes of data more such duplication, neofunctionalization, and recruitment events will be resolved.

Whole genome duplication (WGD) is considered as a potent force for evolution (Ohno, 1970). WGD and its impact on the emergence of evolutionary novelties and complexity, both functional and morphological, are supported by high-throughput sequence data. In vertebrate evolution, there have been two WGD or polyploidization events that are fixed over 500–600 million years (Dehal and Boore, 2005). In snakes, only certain class of toxin genes appear to have duplicated multiple times. For example, in cobras three-finger toxin genes have undergone multiple duplication events (Vonk et al., 2013; Suryamohan et al., 2020; and many transcriptome and proteome studies). In contrast, in crotalids serine protease and metalloprotease genes have undergone multiple duplication events (Yin et al., 2016; Shibata et al., 2018; Schield et al., 2019). Such unusual duplications cannot be explained by WGD.

In addition to WGD, there are four mechanisms of gene duplication. In ectopic recombination, crossing over occurs at non-homologous loci leading to dramatic chromosomal rearrangement, which is generally harmful to the organism. At times, it could lead to chromosomal evolution and rapid speciation (Bush et al., 1977). Transposable elements provide sequence similarity for this non-homologous recombination. The densities of transposable element correlate negatively with ectopic recombination, but the strength of this correlation depends on transposable element (Kent et al., 2017). Replication slippage occurs during DNA replication and produces duplications of short (2–3 bp) genetic sequences. During replication, DNA polymerase dissociates from the DNA and replication stalls. Due to misalignment during reattachment, it copies the same DNA segment again leading to duplication (Viguera et al., 2001). Replication slippage is due to direct repetitive DNA sequences but requires only a few bases of similarity. The slippage efficiency is inversely proportional to the length of the direct repeat (Viguera et al., 2001). Retrotransposition involves reverse transcription of mRNA transcripts to DNA and their insertion in the genome as retrogenes (Naufer et al., 2019). Retrogenes usually lack introns and often contain poly-A sequences. They display changes in gene regulation leading in novel functions. Aneuploidy results in an abnormal number of chromosomes and gene dosage (Birchler et al., 2001). It is detrimental to the organism (Dey, 2004; Newman et al., 2019) and is unlikely to spread through populations. I hypothesize that the ectopic recombination or a distinct new mechanism may explain duplication of toxin genes.

Duplicated genes have three different fates: (a) non-functionalization (pseudogenization) is the most likely fate of duplicated genes based on the assumption that a deleterious mutation is a much more likely outcome than a beneficial mutation (e.g., Dowell et al., 2016); (b) subfunctionalization is a process by which mutations in their regulatory or coding sequences would lead to change in their expression patterns or altered functions, respectively, of the duplicated genes. This can occur because of the relaxation of selection pressures or constraints (e.g., Hargreaves et al., 2014); and (c) neofunctionalization, where one paralogue acquires a new function through mutations that are beneficial for function in regulatory or coding regions, while the other paralogue retains its ancestral function. As beneficial mutations are rare events, neofunctionalization is a rarer event than non-functionalization or subfunctionalization. Mutations in noncoding regulatory regions are more common and plausible, and but mutations in gene-coding regions are rarer. Thus, it is still controversial whether neofunctionalization can indeed be a potent source of evolutionary novelties (For details, see Moriyama and Koshiba-Takeuchi, 2018). In the case of toxins genes, pseudogenization and neofunctionalization are well documented.

The mutations in the regulatory region results in the alteration of expression pattern of duplicated cognate gene from its "parent" tissue to the venom gland. This process is broadly and loosely termed as "recruitment" (Fry and Wüster, 2004; Fry, 2005; Fry et al., 2008). The molecular details in such recruitment have been identified in venom PLA2 and prothrombin activators (Figs. 2–4). Fujimi et al. analyzed several gene sequences encoding PLA2 from *Laticauda semifasciata* to determine the relationship between pancreatic and venom PLA2 genes (Fujimi et al., 2002a, b). They found insertions in the promoter and the first intron of group IA (venom) PLA2 gene compared with group IB" (pancreatic) PLA2 gene (Fujimi et al., 2002a, 2004) (Fig. 2). The 411-bp insert in the promoter region has two E box and one GC box binding sites and interrupts promoter region of pancreatic PLA2 gene (Fig. 2B). AG-rich inserts are ~1100 bp long in venom PLA2 genes compared to 400 bp AG-rich region of pancreatic PLA2 gene (Tamiya and Fujimi, 2006) (Fig. 2A). Similarly, there was one 264 bp insertion in the promoter and 3 insertions and 2 deletions in intron 1 of the *TroD* gene (prothrombin activator gene expressed in the venom gland) compared to the *TrFX* gene (blood coagulation factor X gene expressed in the liver) (Reza et al., 2005, 2007) (Fig. 3). The promoter insert disrupts the *Cis* elements controlling the liver-specific expression and contributes to expression of *TroD* gene in the venom gland. Therefore, we named this insert in *TroD* promoter region as **ve**nom **r**ecruitment/**s**witch **e**lement (*VERSE*) (Reza et al., 2007). The AG-rich regions in second and third inserts in *TroD* intron 1 appear to act as the silencer in restricting its expression to venom glands (Described below) (Fig. 4). These two molecular evidences suggest that inserts in promoter and intron regions alter the tissue-specific expression of duplicated cognate genes from various "parent" tissues to venom gland. In these examples, the promoter inserts are distinct, but contain several *Cis* elements. Such promoter inserts that are responsible for "recruitment" be named as "*Prins*" (Promoter inserts). In contrast, the intron inserts are AG-rich silencers. It will be interesting identify other *Prins* segments and respective silencers responsible for "recruitment" other toxin families. Genomic data will

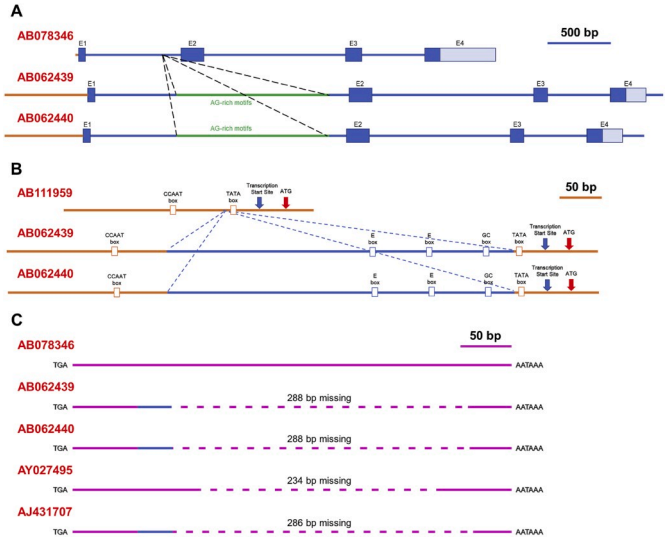

Fig. 2. Comparison of genes encoding pancreatic and venom phospholipase A2 from *Laticauda semifasciata*. **A**. Overview of gene structures of pancreatic PLA2 (AB078346) and venom PLA2s (AB062439 and AB062440). All three genes have four exons. Introns 1 of venom PLA2 genes have several insertions and some of them are AG-rich motifs. Such AG-rich motifs may be responsible for silencing their expression in no-venom gland tissues (Han et al., 2016). **B**. Comparison of promoter regions of pancreatic PLA2 (AM111959) and venom PLA2s (AB062439 and AB062440). The 411-bp inserts found in the venom PLA2 genes compared to pancreatic PLA2 gene. This insert has two E box and one GC box binding sites and contributes to the regulation of expression of the venom PLA2 genes. **C**. Comparison of 3′UTRs of pancreatic PLA2 (AB078346) and venom PLA2s (AB062439 and AB062440). Sequences from 3′UTRs from venom PLA2s from *Pseudonaja textilis* (AY027495) and *Bungarus multicinctus* A2 chain of β-bungarotoxin (AJ431707) are also included. Venom PLA2 genes show deletion of 234–286 bp segments. The effects of this deletion to the stability of venom PLA2 mRNAs is not known.

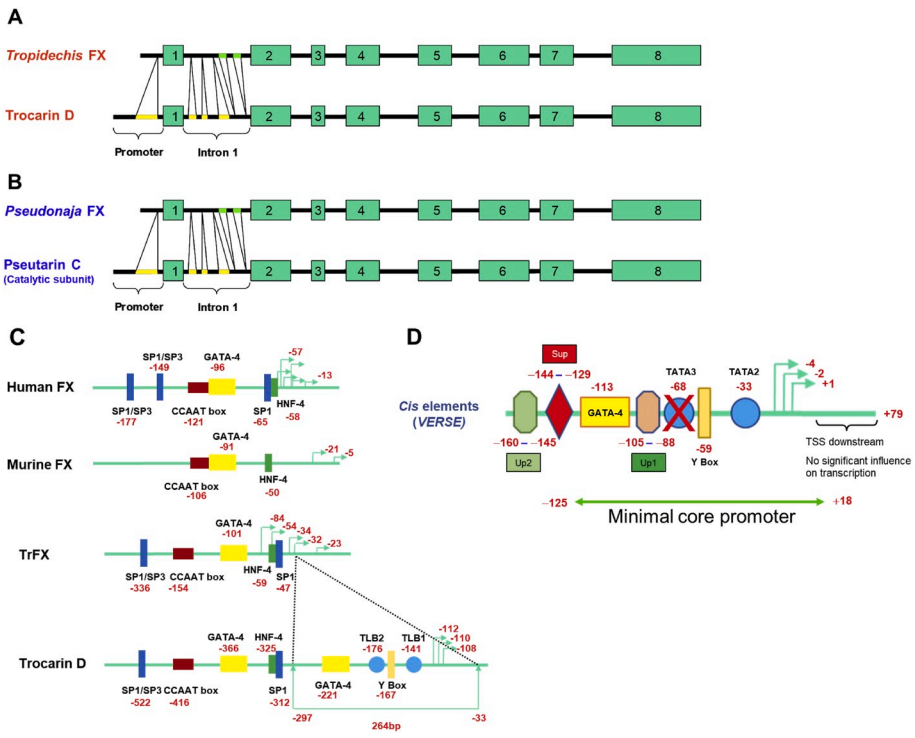

Fig. 3. Comparison of genes encoding liver factor X and venom prothrombin activators. **A**. Liver factor X and venom gland trocarin D genes from *Tropidechis carinatus*. **B**. Liver factor X and venom gland pseutarin C catalytic subunit from *Pseudonaja textilis*. There is a 264-bp inserts, named as *VERSE* (VEnom Recruitment and Switch Element) found in the promoter regions of venom prothrombin activator genes. These genes also have three insertions and two deletions in their introns 1 compared to liver factor X genes. **C**. Comparison of promoter regions of human, mouse and snake liver factor X genes with that of trocarin D. *VERSE* insert has several transcription factor-binding *cis* elements. Similar *VERSE* insert was also found in pseutarin C catalytic subunit. **D**. Identification of minimum core promoter and novel *cis* elements in *VERSE*. By systematic deletion experiments, we identified 135-bp minimum core promoter and two upregulator (Up1 and Up2) and one suppressor (Sup) elements (Kwong et al., 2009).

also help in identifying whether these elements are disrupted through mutations or deletions during reverse "recruitment" (Casewell et al., 2012); in both instances, one could identify the evolutionary trajectory of such cognate genes.

The DNA as well as protein sequence information helps in mutation analyses within the toxin isoforms expressed by a single species, or across multiple species in a genus or even across various genera. Analysis of the cDNA sequences of *Protobothrops* (formerly, *Trimeresurus*) *flavoviridis* (habu snake) venom PLA2 enzymes indicated that the 5′ and 3′ untranslated regions are highly conserved (98% and 89%, respectively) compared to the protein-coding regions (67%) (Ogawa et al., 1992). Further, mutations appeared to have accumulated at similar rates for the three positions of codons. Comparison of the gene sequences indicated that the introns are much more conserved than the protein-coding regions of exons apart from the signal peptide-coding region (Nakashima et al., 1993). The nucleotide substitutions per non-synonymous site (K_A) are close to or larger than mutations at synonymous site (K_S) indicating Darwinian positive selection and accelerated evolution of protein-coding regions. Similar observations in other venom PLA2s, serine proteases and C-type lectin-like proteins suggested that accelerated evolution of exons appears to be universal in toxin isoforms (Nobuhisa et al., 1996; Deshimaru et al., 1996; Ogawa et al., 2005). These accelerated mutations were thought to neofunctionalize the toxins and lead to regional variations with suitable specificity and selectivity towards their target receptor, ion channel or enzyme through positive Darwinian selection (Lynch, 2007; Juárez et al., 2008). Our studies showed that such accelerated mutations appear to target the molecular surface of Class I (elapid) and Class II (crotalid) venom PLA2 enzymes (Kini and Chan, 1999). Interestingly, this adaptive evolution of elapid PLA2 enzymes is associated with speciation events and adaptation of the arsenal to target novel prey species (Lynch, 2007). Using nucleotide sequences of genes for fast-evolving toxins and human hereditary diseases, we showed that specific nucleotide sequences appear to determine point mutation rates (Kini and Chinnasamy, 2010). We classified triplets (not just codons) into stable, unstable and intermediate groups. The relative distribution of stable and unstable triplets is correlated with the accelerated evolution of exons of toxin genes (Kini and Chinnasamy, 2010). An analysis of gene sequences of *Sistrurus catenatus edwardsii* three-finger toxins (3FTxs) indicated that some segments in the exons changed to distinctly different segments compared to corresponding regions of the isoforms (Doley et al., 2008). Such a "switching" of segments in exons may result in drastic alteration in the molecular surface and, hence, the molecular target of these 3FTxs. Thus, we proposed that the phenomenon of **a**ccelerated **s**egment **s**witch in **e**xons to alter **t**argeting (ASSET) may play an important role in the evolution of toxins (Doley et al., 2008). ASSET appears to contribute to functional evolution of 3FTxs to a greater extent compared to other toxin families (Doley et al., 2009). In snake venom serine proteases, ASSET contributes to changes in three surface segments, including the segment near the substrate binding region. Interestingly, these "exchanges" occurred only in the molecular surface of toxins not affecting the structural integrity of toxins (Doley et al., 2008, 2009). Following drastic change through ASSET, accelerated point mutations probably contribute to fine-tuning of target specificity. Evolutionary fingerprint analyses of various groups of 3FTxs indicated that most residues in neurotoxic 3FTxs are under positive Darwinian selection, while κ-neurotoxins and cytotoxic 3FTxs are under negative selection (Sunagar et al., 2013). The authors proposed Rapid Accumulation of Variations in Exposed Residues (RAVERS) or focal mutagenesis are responsible for the functional evolution of 3FTxs. Systematic analyses of venom disintegrin/metalloprotease genes indicated a new mechanism in the evolution through exonization and intronization (Kini, 2018). In the evolution of RTS/KTS disintegrins, a new 34 bp exon (10a) is formed in intron 10. Exon 10a originated from a non-repetitive element, unlike >90% new exons that are from repetitive elements in introns. In this case, simultaneous exonization and intronization occur within a single gene. This new mechanism introduces drastic changes to the molecular surface and alters the function of toxins (Kini, 2018). Increased sequence information combined with systematic analyses will help in fine-tuning some of these mechanisms of accelerated evolution and discover several others.

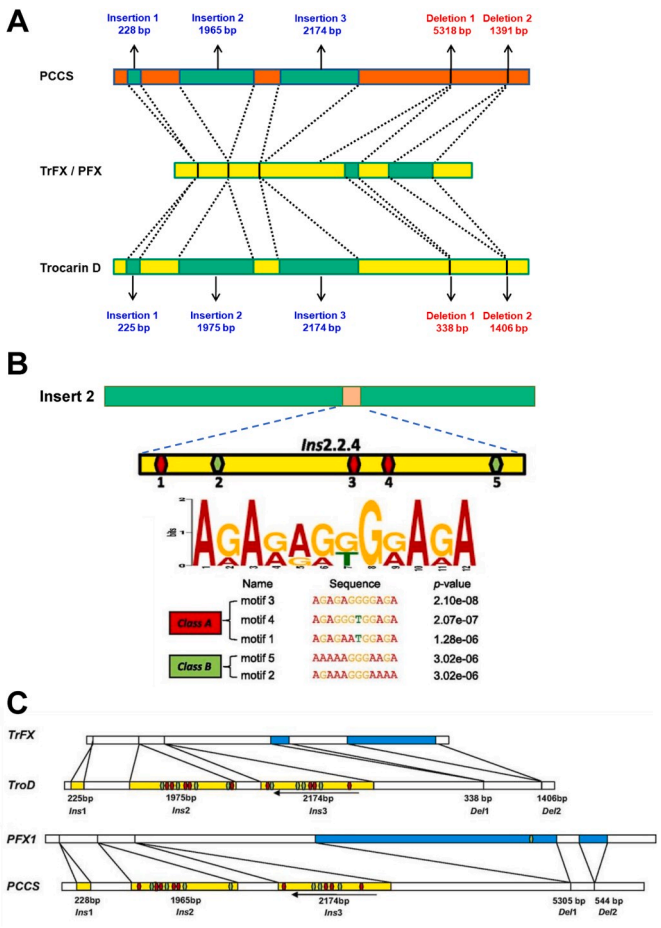

Fig. 4. Identification of silencer elements in venom prothrombin activator genes. **A**. Comparison of introns 1 of liver factor X (TrFX and PFX) and venom prothrombin activator genes (Trocarin D and PCCS, pseutarin C catalytic subunit). Venom gene first introns have three insertions and two deletions compared to their respective cognate genes. **B**. Identification of AG-rich silencer in insert 2. A systematic deletion studies helped us to identify AG-rich motifs as silencers of *VERSE* and *CMV* promoters (for details, see Han et al., 2016). **C**. Distribution of AG-rich motifs in genes encoding trocarin D and PCCS compared to the respective cognate genes. Both inserts 2 and 3 (on the minus strand) have significant number of AG-rich motifs.

4. Toxin expression

Toxins are produced in a specialized gland and their expression is tightly regulated (Kerchove et al., 2004, 2008; Luna et al., 2009; Viana et al., 2017; Yamanouye et al., 2004). Toxin expression is highest when the venom gland is emptied, as in the case of "milking", and it slows down when the gland is full (Rotenberg et al., 1971; Oron and Bdolah, 1973, 1978; De Lucca et al., 1974). It is also apparent in the columnar and squamous stature of the gland epithelial cells that is prominent in crotalid and viperid snakes. Further, the transcriptional activity in vipers shows a sharp increase on Day 3, after a 2-day lag period after milking, and is maintained until 5 days (Paine et al., 1992). In elapid snakes, in contrast, there is no significant change in the morphology of the columnar cells before or after the stimulation of venom production (Lachumanan et al., 1999). The synthesis of nascent toxin mRNAs starts almost immediately (as early as 2 h) after the "milking". In situ hybridization indicated that the CTX, PLA2, and NTX mRNAs are localized only in the columnar secretory epithelial cells. Each secretory cell shows staining for all three toxins. Thus, the toxin gene expression occurs exclusively in these cells and there is no specialization for production of a specific toxin (Lachumanan et al., 1999). The mRNA expression encoding CTX, the most abundant toxin, increases rapidly from 0 to 24 h, while those of PLA2 and NTX increase gradually. The mRNA content for all three toxins are maintained at the elevated levels from two to eight days (Lachumanan et al., 1999). These changes in mRNA content correlates well with the production of respective toxins. Around eight days, once the tubular lumina of the venom glands are filled with venom, toxin synthesis slows to a minimal level (Lachumanan et al., 1999). Interestingly, in viperid snakes it takes about three weeks to reach this equilibrium (Oron and Bdolah, 1978). Such a minimal toxin production status is thought to be due to the enhanced hydrostatic pressure and the amount of venom present in the lumen of the gland, which act as the main regulatory factors for the toxin synthesis (Sells et al., 1989).

In general, toxin genes are selectively expressed in high quantities in the venom gland tissue compared to other tissues including the "parent" tissue. For example, venom group IA PLA2 gene is expressed 100–300 times (based on mRNA contents) higher in the venom gland compared with pancreas, while pancreatic group IB" PLA2 gene is expressed at similar, low levels in both tissues (Fujimi et al., 2004). However, using mass spectrometry the authors could not detect group IB PLA2 in the venom of *L. semifasciata*. Prothrombin activators are also expressed in significantly higher amounts in the venom gland. The expression of trocarin D gene in the venom gland is ~30 times higher compared with that of the TrFX gene in liver (Reza et al., 2007). Similarly, genes encoding pseutarin C catalytic and nonenzymatic subunits are specifically expressed 80- and 280-fold in the venom gland compared to blood coagulation factor X and V genes in liver, respectively (Minh Le et al., 2005; Reza et al., 2006). Such a venom gland-selective expression is due to promoter and other *Cis* elements and corresponding transcription factors.

The TATA box, the first eukaryotic core promoter motif identified (Lifton et al., 1978), regulates expression of 20% genes in yeast (Basehoar et al., 2004) and 24% genes in humans (Yang et al., 2007). It is located 25–35 bp upstream of the transcription start site. Genes containing the TATA-box are more highly regulated when compared to TATA-less genes (Basehoar et al., 2004; Bae et al., 2015). The first gene structure of neurotoxin, that of erabutoxin c, showed the presence of TATA box ($^{-33}$TATAAA^{-28}) in its promoter region (Fuse et al., 1990). Similarly, TATA boxes were found in the promoter regions of snake venom toxin genes, including 3FTx genes (for example, Chang et al., 1997, 1999; Lachumanan et al., 1998; Afifiyan et al., 1999), PLA2 genes (for example, Nakashima et al., 1995; Nobuhisa et al., 1996; Jeyaseelan et al., 2000; Fujimi et al., 2002b; Armugam et al., 2004; Suryamohan et al., 2020), and snake venom serine proteases (for example, Itoh et al., 1988; Reza et al., 2007). Some of these toxin genes have more than one TATA boxes (Lachumanan et al., 1998; Afifiyan et al., 1999; Reza et al., 2007). The role of TATA boxes and associated other *Cis* elements in toxin expression has been characterized in PLA2 genes (Jeyaseelan et al., 2000; Fujimi et al., 2004), 3FTx genes (Gong et al., 2001; Ma et al., 2001, 2002) and prothrombin activator (factor X subunit) gene (Kwong et al., 2009) (Fig. 3C and D). Deletion and site-directed mutation of the above promoters indicate that all these *cis* elements enhance the expression of reporter enzymes in cell culture. Although all TATA boxes are functional, one of them is the strongest promoter element.

As mentioned above, promoter inserts (*Prins*) are responsible for venom gland specific expression and the "recruitment". In the group IA PLA2 (*Laticauda semifasciata* venom LsPLA2GL1-1, LsPLA2GL2-1, and LsPLA2GL5-1) genes, there is a 411 bp insert (*Prins*) between the CCAAT box and TATA box (Fujimi et al., 2002b) compared to the group IB PLA2 (*L. semifasciata* 'pancreatic' LsPLA2GL16-1 and *Protobothrops elegans* pancreatic PLA2 gene) (Fujimi et al., 2004; Chijiwa et al., 2013) (Fig. 2B). This *Prins* segment has one GC box and two E boxes. The full length *Prins* enhances the expression of the reporter gene by ~7-fold (Fujimi et al., 2004). By systematic deletion, the authors identified the segment −232 to −162 (containing both E boxes and GC box) that enhances expression and the segment −410 to −382 (unidentified) that

strongly suppresses reporter gene transcription. Similar *Prins* segments have been found in *Naja sputatrix* (venom acidic and neutral PLA2) (Jeyaseelan et al., 2000), *Bungarus multicinctus* (A1, A2 and A8 chains of β-bungarotoxin) (Chu and Chang, 2002), *Pseudonaja textilis* (venom PLA2 with pancreatic loop Pt-PLA1 and Pt-PLA2) (Armugam et al., 2004), and *Naja naja* (venom PLA2 Nana 39244 and Nana 39246) (Suryamohan et al., 2020) genes. Although the data is incomplete at the 5′ end of *Prins* segments in *N. sputatrix*, *B. multicinctus* and *P. textilis* venom PLA2 genes, this segment varies across different snake genera (data not shown). All *Prins* segments in venom PLA2 genes contain the GC box. Interestingly, one E box is missing in *B. multicinctus* venom PLA2 genes (data not shown). Deletion mutants of *N. sputatrix* promoter showed cell-specific regulation of expression in CHO and HepG2 cells (Jeyaseelan et al., 2000).

The PLA2 *Prins* elements enhanced expression of reporter enzymes by 3- to 7-fold (Jeyaseelan et al., 2000; Fujimi et al., 2004). In contrast, *VERSE* (prothrombin activator *Prins* element) enhances the expression by 19- to 49-fold in HepG2 (liver), HEK293T (kidney) and CHOK1 (ovary) cell lines (Kwong et al., 2009). It also enhanced expression in primary venom gland cells. This 264 bp *VERSE* segment has three TATA boxes (TLB), one GATA-4 and one Y-box. As TLB1 is downstream of the transcription initiation site (TIS), we concluded it as non-functional and mutated all other predicted *cis*-elements. The mutants of the GATA-4, Y-box and TLB2 show 11–51%, 42–70% and 36–62% lower reporter enzyme activity compared to the VERSE promoter, respectively. In contrast, TLB3 mutant shows 14–25% increased activity (Kwong et al., 2009). By a series of deletion mutants, one suppressor and two enhancer *cis* elements were identified (Fig. 3D). Since *VERSE* was able to drive the constitutive expression in non-venom gland cells without any specific induction, it does not contain the *cis*-elements responsible for inducible and tissue-specific expression (Kwong et al., 2009). Subsequently, we showed that AG-rich motifs which act as silencers in intron 1 (Han et al., 2016). These AG-rich motifs are part of two large inserts found in the venom trocarin D and pseutarin C catalytic subunit genes but not in coagulation factor X genes (Reza et al., 2005, 2006) (Fig. 4). Transcription factors YY1, Sp3 and HMGB2 bind to these AG-rich motifs and silence gene expression in mammalian cells. When knockdown these transcription factors, the silencing effects of AG-rich motifs are relieved (Han et al., 2016). Two key features distinguish a silencer from a repressor; a classical silencer exhibits a position-independent repressive activity and it inhibits transcription from heterologous promoters (Ogbourne and Antalis, 1998). As expected of true silencers, constructs containing AG-rich motifs silence heterologous cytomegalovirus (CMV) promoter (Han et al., 2016). AG-rich motifs are found in other toxin (3FTx, PLA2, and metalloprotease) genes but not in their physiologic counterparts (Han et al., 2016). Thus, AG-rich motifs contribute to regulation of expression of toxin genes.

Tissue-specific expression is determined by both *cis*-elements and transcription factors (*Trans* elements). Northern blot studies showed that CTX-2 gene was expressed in venom gland, liver, heart and muscle but not in brain (Ma et al., 2001). Interestingly, the liver, heart and muscle predominantly express ~1 kb mRNAs, while venom gland expresses 0.5 kb mRNA. The liver also expresses a minor transcript of 0.5 kb. Further, transcription initiation and splicing also appears to be affected in various tissues. In the venom gland, there are three TISs – the major TIS is A (+1), while two minor TISs are A (+3) and C (-2) (Ma et al., 2001). In contrast, the major liver-specific TIS is T (-25) and other minor sites are G (-37), G (-98), C (-124) and T (-154). The liver-specific CTX-2 transcripts are lower (~20-times) compared to the venom gland-specific CTX-2 transcripts. In CHO (Chinese Hamster Ovary) cells, although both liver and venom gland-specific TISs are functional, the major TIS is at C (-124). Further, only two CTX-2 transcripts, a full-length 408 bp transcript with 26 bp longer 5′ UTR compared to venom gland transcript and a 289 bp transcript with unspliced intron 1 that encodes truncated product with 21-residue signal peptide and a mature tripeptide (Ma et al., 2001). The latter transcript is the major CTX mRNA in the liver. Although the authors found ~1 kb mRNA in their Northern blot experiments, no transcripts matching this size were obtained during 5′ and 3' rapid amplification of cDNA ends (RACE) analysis (Ma et al., 2001). Thus, the TIS and splicing that affect the expression and function of the protein product, can be different in various tissues. Therefore, careful attention must be paid towards tissue-specific expression, as erroneous observations could be misleading. Systematic analyses of the transcripts along with appropriate quantitative PCR may help resolve some of these issues.

Previously, we evaluated the expression of prothrombin activator and the corresponding coagulation factor genes in the venom glands and liver (Minh Le et al., 2005; Reza et al., 2006, 2007). Venom prothrombin genes are expressed exclusively in the venom gland but not in the liver, while corresponding coagulation factor genes are expressed exclusively in the liver but not in the venom gland. Interestingly, trocarin D promoter and the *VERSE* (a 264 bp *Prins* segment) alone induced expression of reporter enzyme in HepG2, an immortal cell line of polarized human hepatocytes (Kwong et al., 2009). Such a differential expression in the liver and cultured hepatocytes could be due to epigenetic differences the liver DNA compared to newly introduced plasmid DNA. Further, systematic studies may help in understanding the regulation of tissue-specific expression.

Although cardiotoxin and neurotoxin genes are similar, their expression levels are significantly different in the venom gland (Lachumanan et al., 1999). Cardiotoxins account for 60%, while neurotoxins constitute only about 3% of the total proteins in *Naja sputatrix* venom (Tan, 1983). Despite high similarity, the neurotoxin promoter shows several key differences with the cardiotoxin promoter (Ma et al., 2002). The third TATA box (−28 to −33), which is important for the enhanced expression of CTX-2, has A (-32)G mutation. Further, an Ikaros element TCCC is found only in the neurotoxin promoter. The deletion of first 52 bp from the 5' end results in a sharp eight-fold increase in reporter enzyme activity compared to the whole promoter (Ma et al., 2002). This segment shows three single nucleotide substitutions, one nucleotide deletion and one nucleotide insertion compared to corresponding CTX promoter segment. By DNase 1 footprinting and gel retardation assays, the 23 bp segment (−678 to −655) was identified as the silencer (Ma et al., 2002). NTX silencer, in both orientations, inhibits mouse mammary tumor virus promoter and it is 4-fold stronger than CTX silencer (Ma et al., 2002). Systematic evaluation of the existing and newly collected data will help in enhancing our understanding of regulatory processes involved in tissue-specific expression of toxin and related cognate genes.

5. Toxin characterization

As mentioned above, snake venom toxins are structurally and functionally similar to cognate proteins that are expressed in various non-venom gland tissues and play important roles in normal physiological processes. Thus, cognate proteins are essential for our survival. In contrast, with some modification to the structure and accessibility toxins cause devastating debility and death in the prey or victim. Therefore, understanding the structure-function relationships and mechanisms of toxins help us understand the subtle changes that affect their pathophysiological properties.

A classic example for subtle mutation leading to dramatic change is that of sickle cell anemia. Normal red blood cells have a biconcave disc shape. Their elasticity allows them to deform and pass through capillaries. A single point mutation, GAG codon to GTG of the β-globin gene leading to E7V substitution in hemoglobin B subunits. This mutation leads to clumping of hemoglobin and altered structure of red blood cells into sickle cells. These cells attain sickle shape at low oxygen tension. Repeated sickling episodes damage the plasma membrane and reduce the elasticity. These rigid cells fail to deform as they pass through capillaries, leading to vessel occlusion (for a review, see Serjeant, 2010; Carden et al., 2019). Their fragile nature leads to hemolysis and anemia.

The sickled cells last 10–20 days, while normal cells function for 90–120 days. Sickle cell disease causes acute and chronic complications in various organ systems, and the patients suffer from severe pain, anemia, bacterial infections and stroke (Rees et al., 2010; Yawn et al., 2014). The fragile nature of sickle cells provides an adaptive advantage to the heterozygote against malaria infection. Malaria parasite *Plasmodium* fails to reproduce in the red blood cells with defective hemoglobin prematurely rupture. The hemoglobin polymerization also affects its digestion by the parasite. Thus, sickle cell trait increases people's chances of survival in malaria-stricken areas (Kwiatkowski, 2005).

Similar small sequence changes drastically affect structure and function of toxins. Endothelins and sarafotoxins (SRTX), strong vasoconstrictor peptides, are excellent examples. Endothelins are endogenous vasoconstrictor secreted by primarily in the endothelium to maintain the blood pressure (Davenport et al., 2016). Neutral endopeptidase (NEP or Neprilysin), a zinc-dependent metalloprotease cleaves two peptide bonds at the amino side of hydrophobic residues (Ser5-Leu6 followed by Asp18-Ile19) and inactivates endothelins. The removal of C-terminal Trp 21 through the second cleavage inactivates endothelins (Kimura et al., 1988). SRTXs isolated from the venom of *Atractaspis engaddensis* (side-stabbing snakes, stiletto snakes, mole vipers) are structurally and functionally similar to endothelins. Interestingly, SRTXs are resistant to inactivation by neprilysin, probably due to substitutions at both peptide bonds (Skolovsky et al., 1990). Several natural isoforms of SRTXs has been characterized for their function. These studies have helped in understanding subtle structure-function relationships. SRTX-d have two conserved substitutions (Ser2Thr and Val19Ile) compared to SRTX-b (Bdolah et al., 1989). Although both isoforms of sarafotoxins has similar binding affinity to rabbit aorta, SRTX-d weaker vasoconstrictor potency in rabbit aortic strips and lower toxicity compared to SRTXb (Bdolah et al., 1989). Unlike endothelin 1, SRTX S6c distinguishes between endothelin receptor subtypes (Williams et al., 1991). SRTX S6c binds to ET_B subtype found in rat hippocampus and cerebellum with a K_i ~20 pM compared to ET_A subtype found in rat atria and aorta with a K_i ~4500 nM. This selectivity is most likely due to a single substitution Lys9Glu (Takayanagi et al., 1991). Recent crystal structure of SRTX S6b-human endothelin ET_B receptor complex helps in understanding the subtype selectivity of the SRTXs (Izume et al., 2020). Unlike *A. engaddensis* SRTXs, which are 21 residues long, *A. microlepidota microlepidota* SRTXs (SRTX-m) are 24 residues, with three additional residues (DEP) at the C-terminal (Hayashi et al., 2004). In comparison with SRTX-b, SRTX-m shows four orders of magnitude lower affinity for human ET_A and ET_B receptors (Mourier et al., 2012). However, both SRTX-b and SRTX-m have similar in vivo toxicity in mice (Hayashi et al., 2004). Detailed studies indicated that SRTX-b and SRTX-m induce distinct hemodynamic effects in rats (Mahjoub et al., 2015). Although both toxins induce decrease in cardiac output, SRTX-b impairs left ventricle systolic and diastolic function, while SRTX-m induces acute right ventricular dilatation and increase in airway pressures. The truncated SRTX-m without C-terminal DEP shows similar effects as SRTX-b. Thus, the C-terminal DEP contributes significant change in the pharmacologic profile of these closely related toxins (Mahjoub et al., 2015). A simple tripeptide at the C-terminal changes the toxicity of SRTX-b from its vasoconstrictor effect leading to left ventricular failure to the toxicity of SRTX-m from its severe bronchoconstriction effect leading to right ventricular failure.

Akin to SRTXs, natriuretic peptides (NPs) found in snake venom (VNPs) are structural and functional homologues of physiologic NPs that play important roles in blood volume-pressure homeostasis. Human NPs, ANP, BNP and CNP, at low concentrations induce diuresis/natriuresis and at slightly higher concentrations, they also induce vasodilation. All three NPs have a conserved 17-residue NP ring structure, but they differ in their N- and C-terminal segments. Cardiomyocytes secrete ANP and BNP (de Bold, 1985), while vascular endothelial cells secrete CNP (Suga et al., 1993). ANP and BNP activate NPR-A (Waldman et al., 1984), while CNP activates NPR-B (Suga et al., 1992). The ANP/NPR-A signaling regulates vasodilation, increased renal excretion of water and electrolytes, increased endothelial permeability and inhibition of renin-angiotensin-aldosterone system and sympathetic nervous systems (Sasaki et al., 1985; Maack et al., 1986; Brenner et al., 1990). CNP/NPR-B signaling, on the other hand, is mainly involved in tissue remodeling, reproduction and brain functions. It also induces vasodilation and mild hypotensive effects (Chusho et al., 2001). Since the discovery of first NP from *Dendroaspis* venom (DNP; Schweitz et al., 1992), VNPs are found in both elapid and viperid venoms. These VNPs exhibit distinct structural and functional attributes. Compared with ANP, DNP exhibits 60-fold higher resistance to proteolysis and 30-fold lower affinity to NPR-C binding (both involved in clearance of NPs) due to its 14-residue long C-terminal tail (Schweitz et al., 1992). A chimera of CNP and DNP (ring of CNP and C-terminal tail of DNP) called CD-NP has a longer half-life (Dickey et al., 2008; Lisy et al., 2008). CD-NP elicits natriuresis and diuresis through NPR-A activation using its C-terminal tail and vasodilation through NPR-B activation using its NP ring (Dickey et al., 2008). Thus, CD-NP offers the beneficial effects of both NPR-A and NPR-B signaling and avoids severe hypotension by the sole and excessive activation of NPR-A (Zakeri and Burnett, 2011). We identified and characterized a usual krait NP (KNP) from *Bungarus flaviceps* with 38-residue long C-terminal tail, which has the propensity to form an α-helix (Siang et al., 2010; Sridharan and Kini, 2015, 2018). Systematic deletion studies showed that the presence of two pharmacophores that induce vasodilation through orthogonal pathways in KNP: K-Ring elevates intracellular cGMP levels through activation of NPR-A and Helix uses NO-dependent mechanisms (Sridharan and Kini, 2015). Infusion of K-Ring shows transient vasodilation at low doses and sustained vasodilation at high doses in anesthetized rats without affecting urine volume (Sridharan and Kini, 2015, 2018). As K-Ring induced only vasodilation with minimal or no diuretic effects, we used its structure-function relationships to delineate the determinants of hypotensive and diuretic functions in NPs (Sridharan and Kini, 2018). In ANP, conserved residues (F2, M6, D7, R8, I9 and L15) within the 17-membered ring and the C-terminal tail (NSFRY) are crucial for NPR-A binding and in vivo activity (Olins et al., 1988; Li et al., 1995; Ogawa et al., 2004, 2009). Within K-Ring, there are several substitutions (G3D, G4R, G10S, A11T, Q12H and G14D) compared with ANP. As residues at 10, 11 and 12 positions are variable among other NPs, we hypothesized that G3D, G4R and G14D substitutions and the shorter C-terminal tail may be responsible for the distinct functional differences in the hemodynamic and diuretic effects of ANP and K-Ring. By systematic substitution, we evaluated the importance of these three residues within the NP ring and C-terminal on the hemodynamic and diuretic effects in anesthetized rats (Sridharan and Kini, 2018). From these studies, we identified the residues responsible for hypotensive and diuretic effects in K-Ring and transferred these residues in to human ANP to create DGD-ANP, which is a diuretic peptide without vasodilatory function, and DRD-ANP, which is a vasodilatory peptide without diuretic function (Sridharan and Kini, 2018; Sridharan et al., 2020). These peptides differ two or three residues from human ANP, respectively. These peptides were evaluated in conscious normal and heart failure sheep models (Rademaker et al., 2020). In these instrumented sheep models, hemodynamic effects, diuretic effects and neurohormonal changes were monitored after infusion of low, medium and high doses of both these peptides. DGD-ANP induces only diuretic and natriuretic effects, while DRD-ANP induces only hypotensive effects indicating clear separation of pharmacologic profile of these peptides that differ in a single amino acid residue (Rademaker et al., 2020). These first-in-class therapeutic peptides will be useful in the treatment of "Cold and Wet" and "Warm and Wet" acute decompensated heart failure patients (For details, see Sridharan et al., 2020; Rademaker et al., 2020).

There are several other examples of toxins that have 'apparently' small change through substitution/deletion/insertion but exhibit drastic change in functional profiles. Some of these subtle changes also lead to change in disulfide pairing and hence the conformation of the protein

scaffold (Carlier et al., 2001; Kang et al., 2005, 2007; Kang and Kini, 2009; Ng and Kini, 2013; Carbajo et al., 2015).

6. Impact of toxin research

Toxin research has contributed to both fundamental and/or applied aspects of life and biomedical sciences. I will briefly enumerate a small number of examples which could inspire future toxinologists (Fig. 5).

6.1. Research tools

Highly selective interaction of toxins with their respective target receptor, ion channel or enzyme has helped in our understanding of normal physiological processes. α-Bungarotoxin, a neurotoxin isolated from *Bungarus multicinctus* venom (Chang and Lee, 1963) was used as a bait to isolate the nicotinic acetylcholine receptor (nAChR) (Changeux et al., 1970), the first receptor to be isolated and the most thoroughly characterized receptors. Subsequently, α-bungarotoxin and related neurotoxins were used as ideal ligands to probe the structure and localization of various subtypes of nAChRs. Thus, α-neurotoxins have significantly contributed to our understanding on neurotransmission. Botrocetin or venom coagglutinin (Read et al., 1978) is a C-type lectin related protein (snaclecs) from the venom of *Bothrops* species that forms a ternary complex with von Willebrand Factor (vWF) and its platelet receptor GPIb, causing platelet agglutination and aggregation. Thus, botrocetin is an excellent tool for examining interactions between platelets and vWF, for quantification of vWF and for detecting vWF- and GPIb-related diseases (Clemetson et al., 2005; Marsh and Williams, 2005). Another snaclec, convulxin from *Crotalus durissus terrificus* venom (Prado-Franceschi and Brazil, 1981) binds to GPVI one of the two distinct platelet receptors for collagen and induces platelet aggregation (Jandrot-Peruus et al., 1997), while rhodocetin from *Calloselasma rhostoma* venom binds to the second collagen receptor α2β1 and inhibits platelet aggregation (Eble et al., 2001). Thus, snaclecs are used as molecular scalpels in dissecting various stages of platelet aggregation and blood coagulation. For details on snake venom proteins used as research tools in hematology, see (Andrews et al., 2001; Clemetson et al., 2001, 2007; Morita, 2004). Nerve growth factor from *Agkistrodon piscivorus* venom contributed to our knowledge on cell growth regulation (Cohen and Levi-Montalcini, 1956). Stanley Cohen and Rita Levi-Montalcini received Nobel Prize in Physiology and Medicine in 1986 for their work on nerve growth factor and epidermal growth factor. For details, see McCleary and Kini (2013).

6.2. Gene duplication and novel libraries

Gene duplication appears to play critical role in the multiplicity of toxin genes. Depending on the family, genera and species, distinct classes of toxin genes appear to have undergone severe duplications. For example, in elapid and viperid snakes, 3FTx genes and SVMP genes (as well as SVSP genes) have undergone multiple duplication events. In *Pseudechis* genus, PLA2 genes show severe multiple duplications. As of now, it is unclear why only certain toxin genes undergo rampant duplication. If we understand the basic mechanism of duplication, it may be possible to create novel libraries with large number of toxin genes producing extraordinary diversity of toxins. Combining this mode of library creation with accelerated evolution (natural or through error prone polymerases), it will be possible to create unimaginable large libraries of protein toxins in distinct scaffolds.

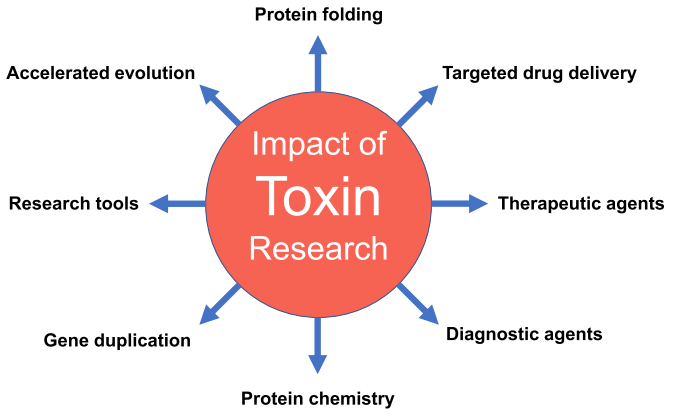

Fig. 5. Overview of impact of toxin research on various areas of life and biomedical sciences.

6.3. Accelerated evolution

Since the first discovery of accelerated evolution of PLA2 genes (Ogawa et al., 1992), it has become clear that almost all toxin genes appear to show accelerated evolution (discussed above). Several theories have been proposed to explain the mechanism of accelerated mechanism. If we successfully understand the mechanism of accelerated evolution, it will aid in the development of novel libraries of protein toxins (described above).

6.4. Protein chemistry

A large number of protein chemistry problems have been resolved through structure-function relationships of toxins. I will highlight three lessons we learnt from toxin research. While studying the structure-function relationships of snake venom PLA2s, we predicted that the myotoxic site consists of a characteristic cationic site next to a hydrophobic region (Kini and Iwanaga, 1986). This cationic site was absent in non-myotoxic PLA2s. Similar characteristic regions of cationic and hydrophobic sites were also found in non-enzymatic myotoxins isolated from snake venoms (Kini and Iwanaga, 1986). We extended this study to cytolysins isolated from phylogenetically unrelated organisms that play either offensive or defensive role (Kini and Evans, 1989a). These pore-forming cytotoxins showed the common features of a cationic site flanked by a hydrophobic surface (Kini and Evans, 1989a). When this positive charge of the cationic site is chemically modified to negative or neutral charges, the cytolytic activity of *Naja nigricollis* cardiotoxin was lost (Kini and Evans, 1989b). When these positive charges are retained by guanidination, cytotoxic activity was retained. Similar cytotoxic sites were identified in other toxins (Chen et al., 1997; Calderón and Lomonte, 1998, 1999). By systematic evaluation of over 1600 protein-protein interaction sites, we showed that proline is most commonly found in the flanking segments. We proposed that the unique chemistry of proline helps protect the integrity and conformation of the interaction sites and present the sites (Kini and Evans, 1995a). These hypothetical structural roles were inspired based on the RGD loop of kistrin (Adler et al., 1991). Based on the presence of proline residues, we developed a simple predictive method to identify protein-protein interaction sites directly from the amino acid sequence (Kini and Evans, 1996). Using this straightforward method, we identified the protein-protein interaction (or functional) sites of toxins (Kini et al., 1998; Srinivasan et al., 2001) and non-toxins (Kini and Evans, 1996; Tan et al., 2001; Venugopal et al., 2018). As proline residues in the flanking segments enhance the protein-protein interactions, we developed a novel method to the design and development of potent peptide drugs (Kini and Evans, 1995b). In this universal method, incorporation of proline residues on both sides of the minimum recognition site enhance the binding ability and concomitant biological activity of the bioactive peptide by 10- to 15-fold through a non-covalent strategy. Thus, using two orthogonal methods (for details, see Kini, 1998) we utilized our finding of the presence of proline residues as the common feature in the flanking segments of protein-protein interaction sites to develop a simple strategy to convert a large, unstable protein in to a small, stable and

easily manipulatable bioactive peptides. Our recent studies on OH9-1 showed that this group of 3FTxs bind to the acetylcholine binding of nAChRs, same site as the classical α-neurotoxins, but with distinct set of functional site residues (Hassan-Puttaswamy et al., 2015). Alanine scan mutations of 12 residues in all three loops of OH9-1 indicated that loop II plays an important role in binding to nAChRs; both sides of loop-II β-strand interact with α1β1εδ, while only one side interacts with α3β2. Although structurally important residues for 3FTx fold are conserved in OH9-1, none of the functionally important residues found in α-neurotoxins are conserved. Thus, this group of toxins, named as Ω-neurotoxins, have evolved independently and are example of unusual convergent (functionally) evolution (Hassan-Puttaswamy et al., 2015). Similarly, Type III neurotoxins (Gong et al., 1999) do not have the functionally important residues found in either α-or Ω-neurotoxins. Subtle changes in the α-neurotoxins leads to interface selectivity with higher affinity for the α-δ site as compared with α-ε or α-γ interfaces (Utkin et al., 2019). Recently, we characterized a novel dimeric neurotoxin, named fulditoxin from *Micrurus fulvius* (Eastern coral snake) (Foo et al., 2020). It has a distinct dimeric quaternary structure and exhibits potent but reversible neurotoxicity in chick biventer cervices muscle. As with all classes of 3FTx neurotoxins described above binds to the acetylcholine binding pocket of nAChRs but shares none of the functional residues. Hence, we named this new group of dimeric toxins, all from *Micrurus* venoms, as Σ-neurotoxins. Thus, all these groups of 3FTx neurotoxins have evolved independently through unusual convergent functional evolution.

6.5. Protein folding

Protein folding is one of the most intriguing fundamental questions of structural biology. Although the amino acid sequence of a protein encodes proper folding and its native three-dimensional structure, the specific determinants involved have not been clearly identified. Disulfide bonds, which are typically non-promiscuous covalent linkages between Cys residues, contribute to correct folding and conformation in disulfide-rich proteins such as toxins. We showed that this high fidelity of correct disulfide linkages in multiple disulfide toxins or protein domains is well-preserved due to a couple of key amino acid residues (Kang et al., 2005, 2007; Ng and Kini, 2013). The globular conformation of α-conotoxins is maintained by the presence of Pro6 in intercysteine loop 1 and the C-terminal amide. Despite conserved Cys residues, χ/λ-conotoxins fold in ribbon conformation due to the replacement of Pro6 by Lys or Ser and the C-terminal amide by free carboxylic acid (For details about these conformational switches, see Kang et al., 2005, 2007). Similarly, a single conserved aromatic, hydrophobic residue in intercysteine loop 3 is key to the formation of hydrophobic core and folding of EGF domains (Ng and Kini, 2013). When this aromatic residue is replaced by hydrophilic residue (Y25T) leads to disruption of the hydrophobic core and EGF fold. Thus, subtle changes in key structural elements alters the disulfide linkages and hence, the disruption of the protein fold.

6.6. Targeted drug delivery

Toxins have high specificity and selectivity towards a specific receptor, ion channel or an enzyme. They breach various barriers in the prey to reach the target tissue or cells. The knowledge of these intricate mechanisms and the protein structures that reaches a specific site in the body will help us develop simple but sophisticated strategies to deliver 'cargoes' to the target tissue. We purified a highly selective neurotoxin, candoxin from *Bungarus candidus* venom that binds reversibly to peripheral nAChR but irreversibly to neuronal α7 nAChR (Nirthanan et al., 2002). Intracerebroventricular injection of candoxin causes apoptosis and death of glial cells, which express α7 nAChR (Pachiappan et al., 2005). Candoxin also induces apoptosis of human glial cells (Hs 683 cells) indicating it is highly specific to glial cells. A 16-mer peptide CDX designed based on the loop II (functional site) of candoxin binds to α7 nAChR as well as primary rat brain capillary endothelial cells (Zhan et al., 2011). CDX labeled micelle carrying paclitaxel extends the survival time of mice with gliobastoma. These vesicles also deliver near infrared fluorescent dye DiR to the brain (Zhan et al., 2011). Using red blood cell derived nanoparticles labeled with ^{D}CDX (synthesized using all D-amino acids with reversed sequence of CDX) created a brain-targeted delivery system for doxorubicin administration (Chai et al., 2017). Thus, based on the candoxin a nAChR-mediated brain drug delivery system was designed. 3FTxs exhibit distinct pharmacological activities by selectively binding to various targets including nAChRs, muscarinic acetylcholine receptors, GABA receptor, adrenergic receptors, $\alpha_{IIb}\beta_3$ integrin, L-type calcium channel, acid-sensitive ion channel, acetylcholinesterase, and several blood coagulation complexes (for details, see Kini, 2002; Fry et al., 2003; Kini and Doley, 2010). In addition, there are at least 20 classes of orphan 3FTxs whose target proteins are yet to be identified (Fry et al., 2003). Similarly, snake venom PLA2 enzymes exhibit a wide variety of pharmacological effects including presynaptic and postsynaptic neurotoxicity, myotoxicity, hemorrhagic activity, anticoagulant and antiplatelet effects due to their specific interaction with various protein targets (For details, see Kini and Evans, 1989c; Kini, 2003). Based on the functional sites of 3FTxs, PLA2s and other toxins, the postal addresses of various tissues or cells, a number of tissue-selective delivery systems can be developed (Kini, 2003). While writing this manuscript, a new drug delivery system that reduces the systemic corticosteroid exposure. By using a cysteine dense particle (CDP) based on a potassium channel blocker from scorpion venom, a highly targeted delivery of arthritis drugs to joints (Cook Sangar et al., 2020). Thus, toxins provide ample opportunity to develop excellent drug delivery systems.

6.7. Diagnostic agents

Based on their effects on blood coagulation and platelet aggregation, several snake venom toxins are being used in diagnosis of hereditary diseases. Some of the standard blood clotting time measurements, such as Stypven time (Hougie, 1956), Ecarin time (Kornalík et al., 1979), dilute Russell viper venom time (Thiagarajan et al., 1986), textarin time (Triplett et al., 1993), Taipan snake venom time (Rooney et al., 1994), and CA-1 method using carinactivase (Iwahashi et al., 2001), are initiated by snake venom factors. These clotting time measurements help in identifying the defects in various blood coagulation factors as well as lupus anticoagulants and oral anticoagulants. Estimation of protein C (Hubbard, 1988), protein S (Han and Pradham, 1990), Factor V Leiden (Sayinalp et al., 2004; Lincz et al., 2006). Similarly, several snake venom proteins affecting platelet aggregation (Andrews et al., 2001; Clemetson et al., 2001, 2007; Morita, 2004) are used to resolve hereditary defects in platelet aggregation. For example, botrocetin is used in understanding the von Willebrand factor and glycoprotein Ib diseases (Brinkhous et al., 1981; Eaton et al., 1991). Similarly, convulxin is used in identifying the deficiency of glycoprotein VI (Nurden et al., 2004) and in developing a highly sensitive method to study platelet aggregation in blood with low platelet counts in patients suffering from Immune thrombocytopenia (ITP) (van Bladel et al., 2014). For details on the use of snake venom proteins in the hematology labs, see reviews (Marsh, 1998, 2001; Marsh and Williams, 2005; Wisner et al., 2001; Schoni, 2005; Perchuc and Wilmer, 2010).

6.8. Therapeutic agents

The toxicity of venoms is one of the main reasons for our fascination with snakes. Over many centuries, scientists focused on understanding this toxicity and the ways to neutralize it. The discovery of bradykinin potentiating peptides in Brazilian pit viper (*Bothrops jararaca*) venom (Ferreira, 1965; Ferreira et al., 1970a, 1970b) followed by the development of non-peptidic inhibitors from peptides led to the synthesis of

captopril (Cushman et al., 1977). This changed the paradigm from "toxin as a villain" to "toxin as a savior". Similarly, discovery of disintegrins from *Trimeresurus gramineus* (Indian green pit viper) and *Echis carinatus* (saw scaled viper) venoms (Huang et al., 1987, 1989; Gan et al., 1988) culminated in the development of potent antiplatelet agents, eptifibatide (Scarborough et al., 1993; Phillips and Scarborough, 1997; Scarborough, 1999) and tirofiban (Barrett et al., 1994; Lynch et al., 1995). Vipegitide, a folded peptidomimetic is being developed as partial antagonist of α2β1 integrin and an antithrombotic agent based on a snaclec from *Vipera palaestinae* venom (Arlinghaus et al., 2013; Momic et al., 2015). As described above, we are developing DRD-ANP and DGD-ANP for the personalized treatment of acute decompensated heart failure patients (Sridharan et al., 2020; Rademaker et al., 2020). There many other laboratories have been working towards developing several novel therapeutic agents for pain, cancer and antimicrobial function. There are several reviews on various aspects of drug development from based on toxins.

7. Conclusions and prospects

In the recent years, Omics technologies have effectively and significantly contributed in obtaining tremendous amount of information in toxinology. It will continue to provide more information in the coming years, with a much lower price tag. The database is expanding in a geometric progression and this growth showing healthy positive exponential. The data available has provided an excellent foundation and opportunities to all toxinologists. Although I have only identified four distinct directions, there are substantially more directions. In each of these directions, finding answers requires multidimensional approaches. These directions are also to an extent interconnected. As of now, we have just started scratching the surface and future expansion will lead us to more interesting questions and is limited only by our imagination. Toxinology provides excellent opportunities for fully intertwined research in fundamental and biomedical sciences. Over the last couple of decades, we have made some inroads but future holds much greater exciting and bright opportunities.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

R. Manjunatha Kini: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing - original draft, Writing - review & editing.

Acknowledgments

I thank thousands of toxinologists who have contributed this field and their contributions helped me guide my own research career. Many of their works, I had the pleasure to read and cite. I apologize to much bigger group of scientists whose work I have not cited here due to my own knowledge limitation combined with the space limitation. I thank my teachers and mentors, who contributed directly: Professors T. Veerabasappa Gowda (University of Mysore, India), Sadaaki Iwanaga (Kyushu University, Japan) and Herbert Evans (Virginia Commonwealth University, USA). I thank Dr. Cassandra Modahl for discussions and reading the manuscript. I thank Ms. Jasmine Jia Min Lim for excellent artwork used in the graphic abstract. I acknowledge the financial support by academic grants by the Ministry of Education, Singapore and the National University of Singapore.

References

Vonk, F.J., Casewell, N.R., Henkel, C.V., Heimberg, A.M., Jansen, H.J., McCleary, R.J., Kerkkamp, H.M., Vos, R.A., Guerreiro, I., Calvete, J.J., Wüster, W., Woods, A.E., Logan, J.M., Harrison, R.A., Castoe, T.A., de Koning, A.P., Pollock, D.D., Yandell, M., Calderon, D., Renjifo, C., Currier, R.B., Salgado, D., Pla, D., Sanz, L., Hyder, A.S., Ribeiro, J.M., Arntzen, J.W., van den Thillart, G.E., Boetzer, M., Pirovano, W., Dirks, R.P., Spaink, H.P., Duboule, D., McGlinn, E., Kini, R.M., Richardson, M.K., 2013. The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. Proc. Natl. Acad. Sci. USA.110 20651–20656. https://doi.org/10.1073/pnas.1314702110. Epub 2013 Dec 2.

Abroug, F., Ouanes-Besbes, L., Tilouche, N., Elatrous, S., 2020. Scorpion envenomation: state of the art. Intensive Care Med. 46, 401–410. https://doi.org/10.1007/s00134-020-05924-8. Epub 2020 Mar 3.

Adler, M., Lazarus, R.A., Dennis, M.S., Wagner, G., 1991. Solution structure of kistrin, a potent platelet aggregation inhibitor and GP IIb-IIIa antagonist. Science 253, 445–448.

Afifiyan, F., Armugam, A., Tan, C.H., Gopalakrishnakone, P., Jeyaseelan, K., 1999. Postsynaptic alpha-neurotoxin gene of the spitting cobra, Naja naja sputatrix: structure, organization, and phylogenetic analysis. Genome Res. 9, 259–266.

Albulescu, L.-O., Hale, M., Ainsworth, S., Alsolaiss, J., Crittenden, E., Calvete, J.J., Wilkinson, M.C., Harrison, R.A., Kool, J., Casewell, N.R., 2019. Preclinical validation of a repurposed metal chelator as a community-based therapeutic for hemotoxic snakebite. BioRxiv doi. https://doi.org/10.1101/717280.

Andrews, R.K., Kamiguti, A.S., Berlanga, O., Leduc, M., Theakston, R.D., Watson, S.P., 2001. The use of snake venom toxins as tools to study platelet receptors for collagen and von Willebrand factor. Haemostasis 31, 155–172.

Arlinghaus, F.T., Momic, T., Ammar, N.A., Shai, E., Spectre, G., Varon, D., Marcinkiewicz, C., Heide, H., Lazarovici, P., Eble, J.A., 2013. Identification of α2β1 integrin inhibitor VP-i with anti-platelet properties in the venom of Vipera palaestinae. Toxicon 64, 96–105.

Armugam, A., Gong, N., Li, X., Siew, P.Y., Chai, S.C., Nair, R., Jeyaseelan, K., 2004. Group IB phospholipase A2 from Pseudonaja textilis. Arch. Biochem. Biophys. 421, 10–20.

Arnold, F.H., 2019. Innovation by evolution: bringing new chemistry to life (Nobel lecture). Angew Chem. Int. Ed. Engl. 58, 14420–14426. https://doi.org/10.1002/anie.201907729. Epub 2019 Aug 21.

Au, L.C., Chou, J.S., Chang, K.J., Teh, G.W., Lin, S.B., 1993. Nucleotide sequence of a full-length cDNA encoding a common precursor of platelet aggregation inhibitor and hemorrhagic protein from Calloselasma rhodostoma venom. Biochim. Biophys. Acta 1173, 243–245.

Bae, S.H., Han, H.W., Moon, J., 2015. Functional analysis of the molecular interactions of TATA box-containing genes and essential genes. PloS One 10, e0120848, 10.1371/journal.pone.0120848.eCollection 2015.

Baedeker, M., Ringel, M., Schulze, U., 2019. FDA approvals hit all time high—but average value slips again. Nat. Rev. Drug Discov. 18, 90. https://doi.org/10.1038/d41573-019-00004-z.

Barrett, J.S., Murphy, G., Peerlinck, K., De Lepeleire, I., Gould, R.J., Panebianco, D., Hand, E., Deckmyn, H., Vermylen, J., Arnout, J., 1994. Pharmacokinetics and pharmacodynamics of MK-383, a selective nonpeptide platelet glycoprotein-IIb/IIIa receptor antagonist, in healthy men. Clin. Pharmacol. Ther. 56, 377–388.

Basehoar, A.D., Zanton, S.J., Pugh, B.F., 2004. Identification and distinct regulation of yeast TATA box-containing genes. Cell 116, 699–709.

Bawaskar, H.S., Bawaskar, P.H., 2019. Snakebite envenoming. Lancet 393, 131. https://doi.org/10.1016/S0140-6736(18)32745-4.

Bdolah, A., Wollberg, Z., Fleminger, G., Kochva, E., 1989. SRTX-d, a new native peptide of the endothelin/sarafotoxin family. FEBS Lett. 256, 1–3.

Birchler, J.A., Bhadra, U., Bhadra, M.P., Auger, D.L., 2001. Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. Dev. Biol. 234, 275–288.

Bjarnason, J.B., Fox, J.W., 1995. Snake venom metalloendopeptidases: reprolysins. Methods Enzymol. 248, 345–368.

Brenner, B.M., Ballermann, B.J., Gunning, M.E., Zeidel, M.L., 1990. Diverse biological actions of atrial natriuretic peptide. Physiol. Rev. 70, 665–699.

Brinkhous, K.M., Read, M.S., Reddick, R.L., Griggs, T.R., 1981. Pathophysiology of platelet-aggregating von Willebrand factor: applications of the venom coagglutinin vWF assay. Ann. N. Y. Acad. Sci. 370, 191–204.

Bryan-Quirós, W., Fernández, J., Gutiérrez, J.M., Lewin, M.R., Lomonte, B., 2019. Neutralizing properties of LY315920 toward snake venom group I and II myotoxic phospholipases A2. Toxicon 157, 1–7. https://doi.org/10.1016/j.toxicon.2018.11.292.

Bulfone, T.C., Samuel, S.P., Bickler, P.E., Lewin, M.R., 2018. Developing small molecule therapeutics for the initial and adjunctive treatment of snakebite. J. Trop. Med. 2018, 4320175, 10.1155/2018/4320175.eCollection 2018.

Bush, G.L., Case, S.M., Wilson, A.C., Patton, J.L., 1977. Rapid speciation and chromosomal evolution in mammals. Proc. Natl. Acad. Sci. U.S.A. 74, 3942–3946.

Calderón, L., Lomonte, B., 1998. Immunochemical characterization and role in toxic activities of region 115-129 of myotoxin II, a Lys 49 phospholipase A2 from Bothrops asper snake venom. Arch. Biochem. Biophys. 358, 343–350.

Calderón, L., Lomonte, B., 1999. Inhibition of the myotoxic activity of Bothrops asper myotoxin II in mice by immunization with its synthetic 13-mer peptide. Toxicon 37, 683–687, 115-129.

Calmette, A., 1894a. Contribution à l'étude du venin des serpents. Immunisation des animaux et traitement de l'envenimation. Ann. Inst. Pasteur. 8, 275–277.

Calmette, A., 1894b. Propriétés du sérum des animaux immunisés contre le venin des serpents et thérapeutique de l'envenimation. C. R. Acad. Sci. 68, 720–722.

Calmette, A., 1896. The treatment of animals poisoned with snake venom by the injection of antivenomous serum. Br. Med. J. 2, 399–400.
Calvete, J.J., 2013. Snake venomics: from the inventory of toxins to biology. Toxicon 75, 44–62. https://doi.org/10.1016/j.toxicon.2013.03.020.
Calvete, J.J., Rodríguez, Y., Quesada-Bernat, S., Pla, D., 2018. Toxin-resolved antivenomics-guided assessment of the immunorecognition landscape of antivenoms. Toxicon 148, 107–122. https://doi.org/10.1016/j.toxicon.2018.04.015.
Carbajo, R.J., Sanz, L., Perez, A., Calvete, J.J., 2015. NMR structure of bitistatin - a missing piece in the evolutionary pathway of snake venom disintegrins. FEBS J. 282, 341–360. https://doi.org/10.1111/febs.13138.
Carden, M.A., Fasano, R.M., Meier, E.R., 2019. Not all red cells sickle the same: contributions of the reticulocyte to disease pathology in sickle cell anemia. Blood Rev. Nov 5, 100637. https://doi.org/10.1016/j.blre.2019.100637 ([Epub ahead of print]).
Carlier, E., Fajloun, Z., Mansuelle, P., Fathallah, M., Mosbah, A., Oughideni, R., Sandoz, G., Di Luccio, E., Geib, S., Regaya, I., Brocard, J., Rochat, H., Darbon, H., Devaux, C., Sabatier, J.M., de Waard, M., 2001. Disulfide bridge reorganization induced by proline mutations in maurotoxin. FEBS Lett. 489, 202–207.
Casewell, N.R., Huttley, G.A., Wuster, W., 2012. Dynamic evolution of venom proteins in squamate reptiles. Nat. Commun. 3, 1066. https://doi.org/10.1038/ncomms2065.
Casewell, N.R., Wüster, W., Vonk, F.J., Harrison, R.A., Fry, B.G., 2013. Complex cocktails: the evolutionary novelty of venoms. Trends Ecol. Evol. 28, 219–229. https://doi.org/10.1016/j.tree.2012.10.020. Epub 2012 Dec 5.
Chai, Z., Hu, X., Wei, X., Zhan, C., Lu, L., Jiang, K., Su, B., Ruan, H., Ran, D., Fang, R.H., Zhang, L., Lu, W., 2017. A facile approach to functionalizing cell membrane-coated nanoparticles with neurotoxin-derived peptide for brain-targeted drug delivery. J. Contr. Release 264, 102–111. https://doi.org/10.1016/j.jconrel.2017.08.027.
Chang, C.C., Lee, C.Y., 1963 Jul. Isolation of neurotoxins from the venom of bungarus multicinctus and their modes of neuromuscular blocking action. Arch. Int. Pharmacodyn. Ther. 1144, 241–257.
Chang, L.S., Lin, J., Chou, Y., Hong, E., 1997. Genomic structure of cardiotoxin 4 and cobratoxin from Naja naja atra (Taiwan cobra). Biochem. Biophys. Res. Commun. 239, 756–762.
Chang, L., Lin, S., Huang, H., Hsiao, M., 1999. Genetic organization of alpha-bungarotoxins from Bungarus multicinctus (Taiwan banded krait): evidence showing that the production of alpha-bungarotoxin isotoxins is not derived from edited mRNAs. Nucleic Acids Res. 27, 3970–3975.
Changeux, J.P., Kasai, M., Lee, C.Y., 1970. Use of a snake venom toxin to characterize the cholinergic receptor protein. Proc. Natl. Acad. Sci. U.S.A. 67, 1241–1247. https://doi.org/10.1073/pnas.67.3.1241.
Chen, K., Arnold, F.H., 1991. Enzyme engineering for nonaqueous solvents: random mutagenesis to enhance activity of subtilisin E in polar organic media. Biotechnol. 9, 1073–1077.
Chen, K., Arnold, F.H., 1993. Tuning the activity of an enzyme for unusual environments: sequential random mutagenesis of subtilisin E for catalysis in dimethylformamide. Proc. Natl. Acad. Sci. U.S.A. 90, 5618–5622.
Chen, D., Kini, R.M., Yuen, R., Khoo, H.E., 1997. Haemolytic activity of stonustoxin from stonefish (Synanceja horrida) venom: pore formation and the role of cationic amino acid residues. Biochem. J. 325, 685–691.
Chijiwa, T., Nakasone, H., Irie, S., Ikeda, N., Tomoda, K., Oda-Ueda, N., Hattori, S., Ohno, M., 2013. Structural characteristics and evolution of the Protobothrops elegans pancreatic phospholipase A2 gene in contrast with those of Protobothrops genus venom phospholipase A2 genes. Biosci. Biotechnol. Biochem. 77, 97–102.
Chippaux, J.P., Goyffon, M., 2008. Epidemiology of scorpionism: a global appraisal. Acta Trop. 107, 71–79. https://doi.org/10.1016/j.actatropica.2008.05.021. Epub 2008 Jun 5.
Chu, Y.P., Chang, L.S., 2002. The organization of the genes encoding the A chains of beta-bungarotoxins: evidence for the skipping of exon. Toxicon 40, 1437–1443.
Chusho, H., Tamura, N., Ogawa, Y., Yasoda, A., Suda, M., Miyazawa, T., Nakamura, K., Nakao, K., Kurihara, T., Komatsu, Y., Itoh, H., Tanaka, K., Saito, Y., Katsuki, M., Nakao, K., 2001. Dwarfism and early death in mice lacking C-type natriuretic peptide. Proc. Natl. Acad. Sci. U.S.A. 98, 4016–4021. https://doi.org/10.1073/pnas.071389098.
Clemetson, K.J., Navdaev, A., Dörmann, D., Du, X.Y., Clemetson, J.M., 2001. Multifunctional snake C-type lectins affecting platelets. Haemostasis 31, 148–154.
Clemetson, K.J., Lu, Q., Clemetson, J.M., 2005. Snake C-type lectin-like proteins and platelet receptors. Pathophysiol. Haemostasis Thrombosis 34, 150–155.
Clemetson, K.J., Lu, Q., Clemetson, J.M., 2007. Snake venom proteins affecting platelets and their applications to anti-thrombotic research. Curr. Pharmaceut. Des. 13, 2887–2892.
Cohen, S., Levi-Montalcini, R., 1956. A nerve growth-stimulating factor isolated from snake venom. Proc. Natl. Acad. Sci. U.S.A. 42, 571–574.
Cook Sangar, M.L., Girard, E.J., Hopping, G., Yin, C., Pakiam, F., Brusniak, M.Y., Nguyen, E., Ruff, R., Gewe, M.M., Byrnes-Blake, K., Nairn, N.W., Miller, D.M., Mehlin, C., Strand, A.D., Mhyre, A.J., Correnti, C.E., Strong, R.K., Simon, J.A., Olson, J.M., 2020. A potent peptide-steroid conjugate accumulates in cartilage and reverses arthritis without evidence of systemic corticosteroid exposure. Sci. Transl. Med. 12, eaay1041 https://doi.org/10.1126/scitranslmed.aay1041.
Cousin, X., Bon, S., Massoulie, J., Bon, C., 1998. Identification of a novel type of alternatively spliced exon from the acetylcholinesterase gene of Bungarus fasciatus. Molecular forms of acetylcholinesterase in the snake liver and muscle. J. Biol. Chem. 273, 9812–9830.
Cushman, D.W., Cheung, H.S., Sabo, E.F., Ondetti, M.A., 1977. Design of potent competitive inhibitors of angiotensin-converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids. Biochemistry 16, 5484–5491.
Davenport, A.P., Hyndman, K.A., Dhaun, N., Southan, C., Kohan, D.E., Pollock, J.S., Pollock, D.M., Webb, D.J., Maguire, J.J., 2016. Endothelin. Pharmacol. Rev. 68, 357–418.
de Bold, A.J., 1985. Atrial natriuretic factor: a hormone produced by the heart. Science 230, 767–770. https://doi.org/10.1126/science.2932797.
De Lucca, F.L., Haddad, A., Kochva, E., Rothschild, A.M., Valeri, V., 1974. Protein synthesis and morphological changes in the secretory epithelium of venom gland of Crotalus durissus terrificus at different times after manual extraction of venom. Toxicon 12, 361–368.
Dehal, P., Boore, J.L., 2005. Two rounds of whole genome duplication in the ancestral vertebrate. PLoS Biol. 3, e314.
Deshimaru, M., Ogawa, T., Nakashima, K., Nobuhisa, I., Chijiwa, T., Shimohigashi, Y., Fukumaki, Y., Niwa, M., Yamashina, I., Hattori, S., Ohno, M., 1996. Accelerated evolution of crotalinae snake venom gland serine proteases. FEBS Lett. 397, 83–88.
Dey, P., 2004. Aneuploidy and malignancy: an unsolved equation. J. Clin. Pathol. 57, 1245–1249.
Dickey, D.M., Burnett Jr., J.C., Potter, L.R., 2008. Novel bifunctional natriuretic peptides as potential therapeutics. J. Biol. Chem. 283, 35003–35009. https://doi.org/10.1074/jbc.M804538200.
Doley, R., Pahari, S., Mackessy, S.P., Kini, R.M., 2008. Accelerated exchange of exon segments in Viperid three-finger toxin genes (Sistrurus catenatus edwardsii; Desert Massasauga). BMC Evol. Biol. 8, 196. https://doi.org/10.1186/1471-2148-8-196.
Doley, R., Mackessy, S.P., Kini, R.M., 2009. Role of accelerated segment switch in exons to alter targeting (ASSET) in the molecular evolution of snake venom proteins. BMC Evol. Biol. 9, 146. https://doi.org/10.1186/1471-2148-9-146.
Dowell, N.L., Giorgianni, M.W., Kassner, V.A., Selegue, J.E., Sanchez, E.E., Carroll, S.B., 2016. The deep origin and recent loss of venom toxin genes in rattlesnakes. Curr. Biol. 26, 2434–2445. https://doi.org/10.1016/j.cub.2016.07.038. Epub 2016 Sep. 15.
Eaton Jr., L.A., Read, M.S., Brinkhous, K.M., 1991. Glycoprotein Ib bioassays. Activity levels in Bernard-Soulier syndrome and in stored blood bank platelets. Arch. Pathol. Lab Med. 115, 488–493.
Eble, J.A., Beermann, B., Hinz, H.J., Schmidt-Hederich, A., 2001. alpha2beta1 integrin is not recognized by rhodocytin but is the specific, high affinity target of rhodocetin, an RGD-independent disintegrin and potent inhibitor of cell adhesion to collagen. J. Biol. Chem. 276, 12274–12284.
Ferreira, S.H., 1965. A Bradykinin-potentiation factor (BPF) present in the venom of Bothrops jararaca. Br. J. Pharmacol. 24, 163–169.
Ferreira, S.H., Bartelt, D.C., Greene, L.J., 1970a. Isolation of bradykinin-potentiating peptides from Bothrops jararaca venom. Biochemistry 9, 2583–2593.
Ferreira, S.H., Greene, L.H., Alabaster, V.A., Bakhle, Y.S., Vane, J.R., 1970b. Activity of various fractions of bradykinin potentiating factor against angiotensin I converting enzyme. Nature 225, 379–380.
Fleming, T.J., OhUigin, C., Malek, T.R., 1993. Characterization of two novel Ly-6 genes. Protein sequence and potential structural similarity to a-bungarotoxin and other neurotoxins. J. Immunol. 150, 5379–5390.
Foo, C.S., Jobichen, C., Hassan-Puttaswamy, V., Dekan, Z., Tae, H.S., Bertrand, D., Adams, D.J., Alewood, P.F., Sivaraman, J., Nirthanan, S., Kini, R.M., 2020. Fulditoxin, representing a new class of dimeric snake toxins, defines novel pharmacology at nicotinic ACh receptors. Br. J. Pharmacol. 177, 1822–1840. https://doi.org/10.1111/bph.14954. Epub 2020 Feb 9.
Fry, B.G., 2005. From genome to "venome": molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. Genome Res. 15, 403–420.
Fry, B.G., Wüster, W., 2004. Assembling an arsenal: origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences. Mol. Biol. Evol. 21, 870–883.
Fry, B.G., Wüster, W., Kini, R.M., Brusic, V., Khan, A., Venkataraman, D., Rooney, A.P., 2003. Molecular evolution and phylogeny of elapid snake venom three finger toxins. J. Mol. Evol. 57, 110–129.
Fry, B.G., Scheib, H., van der Weerd, L., Young, B., McNaughtan, J., Ramjan, S.F.R., Vidal, N., Poelmann, R.E., Norman, J.A., 2008. Evolution of an arsenal: structural and functional diversification of the venom system in the advanced snakes (Caenophidia). Mol. Cell. Proteomics 7, 215–246.
Fry, B.G., Roelants, K., Champagne, D.E., Scheib, H., Tyndall, J.D.A., King, G.F., Nevalainen, T.J., Norman, J.A., Lewis, R.J., Norton, R.S., Renjifo, C., de la Vega, R. C., 2009. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu. Rev. Genom. Hum. Genet. 10, 483–511. https://doi.org/10.1146/annurev.genom.9.081307.164356.
Fujimi, T.J., Kariya, Y., Tsuchiya, T., Tamiya, T., 2002a. Nucleotide sequence of phospholipase A(2) gene expressed in snake pancreas reveals the molecular evolution of toxic phospholipase A(2) genes. Gene 292, 225–231.
Fujimi, T.J., Tsuchiya, T., Tamiya, T., 2002b. A comparative analysis of invaded sequences from group IA phospholipase A(2) genes provides evidence about the divergence period of genes groups and snake families. Toxicon 40, 873–884.
Fujimi, T.J., Yasuoka, S., Ogura, E., Tsuchiya, T., Tamiya, T., 2004. Comparative analysis of gene expression mechanisms between group IA and IB phospholipase A2 genes from sea snake Laticauda semifasciata. Gene 332, 179–190.
Fuse, N., Tsuchiya, T., Nonomura, Y., Menez, A., Tamiya, T., 1990. Structure of the snake short-chain neurotoxin, erabutoxin c, precursor gene. Eur. J. Biochem. 193, 629–633.
Gan, Z.R., Gould, R.J., Jacobs, J.W., Friedman, P.A., Polokoff, M.A., 1988. Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, Echis carinatus. J. Biol. Chem. 263, 19827–19832.

Gong, N., Armugam, A., Jeyaseelan, K., 1999. Postsynaptic short-chain neurotoxins from Pseudonaja textilis. cDNA cloning, expression and protein characterization. Eur. J. Biochem. 265, 982–989.
Gong, N., Armugam, A., Mirtschin, P., Jeyaseelan, K., 2001. Cloning and characterization of the pseudonajatoxin b precursor. Biochem. J. 358, 647–656.
Gutiérrez, J.M., León, G., Lomonte, B., Angulo, Y., 2011. Antivenoms for snakebite envenomings. Inflamm. Allergy - Drug Targets 10, 369–380.
Gutiérrez, J.M., Calvete, J.J., Habib, A.G., Harrison, R.A., Williams, D.J., Warrell, D.A., 2017. Snakebite envenoming. Nat. Rev. Dis. Primers 2017 (3), 17063. https://doi.org/10.1038/nrdp.2017.63.
Halpert, J., Eaker, D., 1975. Amino acid sequence of a presynaptic neurotoxin from the venom of Notechis scutatus scutatus (Australian tiger snake). J. Biol. Chem. 250, 6990–6997.
Han, P., Pradham, M., 1990. A simple functional protein S assay using PROTAC. Clin. Lab. Haematol. 12, 201–208.
Han, S.X., Kwong, S., Ge, R., Kolatkar, P.R., Woods, A.E., Blanchet, G., Kini, R.M., 2016. Regulation of expression of venom toxins: silencing of prothrombin activator trocarin D by AG-rich motifs. Faseb. J. 30, 2411–2425. https://doi.org/10.1096/fj.201600213R. Epub 2016 Mar 16.
Hargreaves, A.D., Swain, M.T., Hegarty, M.J., Logan, D.W., Mulley, J.F., 2014. Restriction and recruitment-gene duplication and the origin and evolution of snake venom toxins. Genome Biol. Evol. 6, 2088–2095. https://doi.org/10.1093/gbe/evu166.
Hassan-Puttaswamy, V., Adams, D.J., Kini, R.M., 2015. A distinct functional site in Ω-neurotoxins: novel antagonists of nicotinic acetylcholine receptors from snake venom. ACS Chem. Biol. 10, 2805–2815. https://doi.org/10.1021/acschembio.5b00492. Epub 2015 Oct 15.
Hayashi, M.A.F., Ligny-Lemaire, C., Wollberg, Z., Wery, M., Galat, A., Ogawa, T., Muller, B.H., Lamthanh, H., Doljansky, Y., Bdolah, A., Stöcklin, R., Ducancel, F., 2004. Long-sarafotoxins: characterization of a new family of endothelin-like peptides. Peptides 25, 1243–1251.
Heinrikson, R.L., Krueger, E.T., Keim, P.S., 1977. Amino acid sequence of phospholipase A2-alpha from the venom of Crotalus adamanteus. A new classification of phospholipases A2 based upon structural determinants. J. Biol. Chem. 252, 4913–4921.
Herrera, M., Paiva, O.K., Pagotto, A.H., Segura, A., Serrano, S.M.T., Vargas, M., Villalta, M., Jensen, S.D., León, G., Williams, D.J., Gutiérrez, J.M., 2014. Antivenomic characterization of two antivenoms against the venom of the taipan, Oxyuranus scutellatus, from Papua New Guinea and Australia. Am. J. Trop. Med. Hyg. 91, 887–894.
Hite, L.A., Shannon, J.D., Bjarnason, J.B., Fox, J.W., 1992. Sequence of a cDNA clone encoding the zinc metalloproteinase hemorrhagic toxin e from Crotalus atrox: evidence for signal, zymogen, and disintegrin-like structures. Biochemistry 31, 6203–6211.
Hite, L.A., Bjarnason, J.B., Fox, J.W., 1994. cDNA sequences for four snake venom metalloproteinases: structure, classification, and their relationship to mammalian reproductive proteins. Arch. Biochem. Biophys. 308, 182–191.
Hooft van Huijsduijnen, R., Kojima, S., Carter, D., Okabe, H., Sato, A., Akahata, W., Wells, T.N.C., Katsuno, K., 2020. Reassessing therapeutic antibodies for neglected and tropical diseases. PLoS Neglected Trop. Dis. 14, e0007860, 10.1371/journal.pntd.0007860.eCollection 2020 Jan.
Hoogenboom, H.R., de Bruïne, A.P., Hufton, S.E., Hoet, R.M., Arends, J.-W., Roovers, R.C., 1998. Antibody phage display technology and its applications. Immunotechnology 4, 1–20.
Hooks, M.A., Wade, C.S., Millikan, W.J., 1991. Muromonab CD-3: a review of its pharmacology, pharmacokinetics, and clinical use in transplantation. Pharmacotherapy 11, 26–37.
Hougie, C., 1956. Effect of Russell's viper venom (Stypven) on Stuart clotting defect. Proc. Soc. Exp. Biol. Med. 98, 570–573.
Huang, T.F., Holt, J.C., Lukasiewicz, H., Niewiarowski, S., 1987. Trigramin. A low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb-IIIa complex. J. Biol. Chem. 262, 16157–16163.
Huang, T.F., Holt, J.C., Kirby, E.P., Niewiarowski, S., 1989. Trigramin: primary structure and its inhibition of von Willebrand factor binding to glycoprotein IIb/IIIa complex on human platelets. Biochemistry 28, 661–666.
Hubbard, A.R., 1988. Standardization of protein C in plasma: establishment of an international standard. Thromb. Haemostasis 59, 464–467.
Hunter, C.J., Piechazek, K.H., Nyarang'o, P.M., Rennie, T., 2019. Snakebite envenoming. Lancet 393, 129–131. https://doi.org/10.1016/S0140-6736(18)32762-4.
Inoue, S., Oda, T., Koyama, J., Ikeda, K., Hayashi, K., 1991. Amino acid sequences of nerve growth factors derived from cobra venoms. FEBS Lett. 279, 38–40.
Itoh, N., Tanaka, N., Funakoshi, I., Kawasaki, T., Mihashi, S., Yamashina, I., 1988. Organization of the gene for batroxobin, a thrombin-like snake venom enzyme. Homology with the trypsin/kallikrein gene family. J. Biol. Chem. 263, 7628–7631.
Iwahashi, H., Kimura, M., Nakajima, K., Yamada, D., Morita, T., 2001. Determination of plasma prothrombin level by Ca2+-dependent prothrombin activator (CA-1) during warfarin anticoagulation. J. Heart Valve Dis. 10, 388–392.
Izume, T., Miyauchi, H., Shihoya, W., Nureki, O., 2020. Crystal structure of human endothelin ETB receptor in complex with sarafotoxin S6b. Biochem. Biophys. Res. Commun. 20, 30021–30028. https://doi.org/10.1016/j.bbrc.2019.12.091. Jan 28. pii: S0006-291X, ([Epub ahead of print]).
Jandrot-Peruus, M., Lagrue, A.-H., Okuma, M., Bon, C., 1997. Adhesion and activation of human platelets induced by convulxin involve glycoprotein VI and integrin α2β1. J. Biol. Chem. 272, 27035–27041.
Jeyaseelan, K., Armugam, A., Ma, D., Tan, N.H., 2000. Structure and phylogeny of the venom group I phospholipase A(2) gene. Mol. Biol. Evol. 17, 1010–1021.
Jia, L.G., Shimokawa, K., Bjarnason, J.B., Fox, J.W., 1996. Snake venom metalloproteinases: structure, function and relationship to the ADAMs family of proteins. Toxicon 34, 1269–1276.
Joseph, J.S., Chung, M.C., Jeyaseelan, K., Kini, R.M., 1999. Amino acid sequence of trocarin, a prothrombin activator from Tropidechis carinatus venom: its structural similarity to coagulation factor Xa. Blood 94, 621–631.
Juárez, P., Comas, I., González-Candelas, F., Calvete, J.J., 2008. Evolution of snake venom disintegrins by positive Darwinian selection. Mol. Biol. Evol. 25, 2391–2407. https://doi.org/10.1093/molbev/msn179. Epub 2008 Aug 13.
Kang, T.S., Kini, R.M., 2009. Structural determinants of protein folding. Cell. Mol. Life Sci. 66, 2341–2361. https://doi.org/10.1007/s00018-009-0023-5. Epub 2009 Apr 15.
Kang, T.S., Vivekanandan, S., Jois, S.D., Kini, R.M., 2005. Effect of C-terminal amidation on folding and disulfide-pairing of alpha-conotoxin ImI. Angew Chem. Int. Ed. Engl. 44, 6333–6337.
Kang, T.S., Radić, Z., Talley, T.T., Jois, S.D., Taylor, P., Kini, R.M., 2007. Protein folding determinants: structural features determining alternative disulfide pairing in alpha- and chi/lambda-conotoxins. Biochemistry 46, 3338–3355. Epub 2007 Feb 22.
Kaplon, H., Reichert, J.M., 2019. Antibodies to watch in 2019. mAbs 11, 219–238. https://doi.org/10.1080/19420862.2018.1556465. Epub 2018 Dec 22.
Kaplon, H., Muralidharan, M., Schneider, Z., Reichert, J.M., 2020. Antibodies to watch in 2020. mAbs 12 (1), 1703531. https://doi.org/10.1080/19420862.2019.1703531, 2020 Jan-Dec.
Kent, T.V., Uzunović, J., Wright, S.I., 2017. Coevolution between transposable elements and recombination. Philos. Trans. R. Soc. Lond. B Biol. Sci. 372, 20160458. https://doi.org/10.1098/rstb.2016.0458 pii.
Kerchove, C.M., Carneiro, S.M., Markus, R.P., Yamanouye, N., 2004. Stimulation of the alpha-adrenoceptor triggers the venom production cycle in the venom gland of Bothrops jararaca. J. Exp. Biol. 207, 411–416.
Kerchove, C.M., Luna, M.S., Zablith, M.B., Lazari, M.F., Smaili, S.S., Yamanouye, N., 2008. Alpha1-adrenoceptors trigger the snake venom production cycle in secretory cells by activating phosphatidylinositol 4,5-bisphosphate hydrolysis and ERK signaling pathway. Comp. Biochem. Physiol. Mol. Integr. Physiol. 150, 431–437. https://doi.org/10.1016/j.cbpa.2008.04.607.
Kimura, S., Kasuya, Y., Sawamura, T., Shinmi, O., Sugita, Y., Yanagisawa, M., Goto, K., Masaki, T., 1988. Structure-activity relationships of endothelin: importance of the C-terminal moiety. Biochem. Biophys. Res. Commun. 156, 1182–1186.
Kini, R.M., 1998. Proline brackets and identification of potential functional sites in proteins: toxins to therapeutics. Toxicon 36, 1659–1670.
Kini, R.M., 2002. Molecular molds with multiple missions: functional sites in three-finger toxins. Clin. Exp. Pharmacol. Physiol. 29, 815–822.
Kini, R.M., 2003. Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. Toxicon 42, 827–840, 2003.
Kini, R.M., 2018. Accelerated evolution of toxin genes: exonization and intronization in snake venom disintegrin/metalloprotease genes. Toxicon 148, 16–25. https://doi.org/10.1016/j.toxicon.2018.04.005. Epub 2018 Apr 7.
Kini, R.M., Chan, Y.M., 1999. Accelerated evolution and molecular surface of venom phospholipase A2 enzymes. J. Mol. Evol. 48, 125–132.
Kini, R.M., Chinnasamy, A., 2010. Nucleotide sequence determines the accelerated rate of point mutations. Toxicon 56, 295–304. https://doi.org/10.1016/j.toxicon.2009.12.022. Epub 2010 Apr 1.
Kini, R.M., Doley, R., 2010. Structure, function and evolution of three-finger toxins: mini proteins with multiple targets. Toxicon 56, 855–867. https://doi.org/10.1016/j.toxicon.2010.07.010.
Kini, R.M., Evans, H.J., 1989a. A common cytolytic region in myotoxins, hemolysins, cardiotoxins and antibacterial peptides. Int. J. Pept. Protein Res. 34, 277–286.
Kini, R.M., Evans, H.J., 1989b. Role of cationic residues in cytolytic activity: modification of lysine residues in the cardiotoxin from Naja nigricollis venom and correlation between cytolytic and antiplatelet activity. Biochemistry 28, 9209–9215.
Kini, R.M., Evans, H.J., 1989c. A model to explain the pharmacological effects of snake venom phospholipases A2. Toxicon 27, 613–635.
Kini, R.M., Evans, H.J., 1995a. A hypothetical structural role for proline residues in the flanking segments of protein-protein interaction sites. Biochem. Biophys. Res. Commun. 212, 1115–1124.
Kini, R.M., Evans, H.J., 1995b. A novel approach to the design of potent bioactive peptides by incorporation of proline brackets: antiplatelet effects of Arg-Gly-Asp peptides. FEBS Lett. 375, 15–17.
Kini, R.M., Evans, H.J., 1996. Prediction of potential protein-protein interaction sites from amino acid sequence. Identification of a fibrin polymerization site. FEBS Lett. 385, 81–86.
Kini, R.M., Iwanaga, S., 1986. Structure-function relationships of phospholipases. II: charge density distribution and the myotoxicity of presynaptically neurotoxic phospholipases. Toxicon 24, 895–905.
Kini, R.M., Caldwell, R.A., Wu, Q.Y., Baumgarten, C.M., Feher, J.J., Evans, H.J., 1998. Flanking proline residues identify the L-type Ca2+ channel binding site of calciseptine and FS2. Biochemistry 37, 9058–9063.
Kini, R.M., Sidhu, S.S., Laustsen, A.H., 2018. Biosynthetic oligoclonal antivenom (BOA) for snakebite and next-generation treatments for snakebite victims. Toxins 10. https://doi.org/10.3390/toxins10120534 pii: E534.
Klutz, S., Holtmann, L., Lobedann, M., Schembecker, G., 2016. Cost evaluation of antibody production processes in different operation modes. Chem. Eng. Sci. 141, 63–74.
Kochva, E., Viljoen, C.C., Botes, D.P., 1982. A new type of toxin in the venom of snakes of the genus Atractaspis (Atractaspidinae). Toxicon 20, 581–592.
Kochva, E., Bdolah, A., Wollberg, Z., 1993. Sarafotoxins and endothelins: evolution, structure and function. Toxicon 31, 541–568.

Komori, Y., Sugihara, H., 1990. Purification and physiological study of a hypotensive factor from the venom of Vipera aspis aspis (aspic viper). Toxicon 28, 359–369.
Komori, Y., Nikai, T., Taniguchi, K., Masuda, K., Sugihara, H., 1999. Vascular endothelial growth factor VEGF-like heparin-binding protein from the venom of Vipera aspis aspis (Aspic viper). Biochemistry 38, 11796–11803.
Kornalík, F., Dyr, J.E., Vodrázka, Z., Fortová, H., 1979. Fibrinogenolytic effect of ecarin a prothrombin converting enzyme. Thromb. Res. 15, 27–36.
Kostiza, T., Meier, J., 1996. Nerve growth factors from snake venoms: chemical properties, mode of action and biological significance. Toxicon 34, 787–806.
Kwiatkowski, D.P., 2005. How malaria has affected the human genome and what human genetics can teach us about malaria. Am. J. Hum. Genet. 77, 171–192.
Kwong, S., Woods, A.E., Mirtschin, P.J., Ge, R., Kini, R.M., 2009. The recruitment of blood coagulation factor X into snake venom gland as a toxin: the role of promoter cis-elements in its expression. Thromb. Haemostasis 102, 469–478. https://doi.org/10.1160/TH09-03-0162.
Lachumanan, R., Armugam, A., Tan, C.H., Jeyaseelan, K., 1998. Structure and organization of the cardiotoxin genes in Naja naja sputatrix. FEBS Lett. 433, 119–124.
Lachumanan, R., Armugam, A., Durairaj, P., Gopalakrishnakone, P., Tan, C.H., Jeyaseelan, K., 1999. In situ hybridization and immunohistochemical analysis of the expression of cardiotoxin and neurotoxin genes in Naja naja sputatrix. J. Histochem. Cytochem. 47, 551–560.
Lalloo, D.G., Theakston, R.D.G., 2003. Snake antivenoms. J. Toxicol. Clin. Toxicol. 41, 277–290.
Laustsen, A.H., Dorrestijn, N., 2018. Integrating engineering, manufacturing, and regulatory considerations in the development of novel antivenoms. Toxins 10, 309.
Laustsen, A.H., Karatt-Vellatt, A., Masters, E.W., Arias, A.S., Pus, U., Knudsen, C., Oscoz, S., Slavny, P., Griffiths, D.T., Luther, A.M., Leah, R.A., Lindholm, M., Lomonte, B., Gutiérrez, J.M., McCafferty, J., 2018. In vivo neutralization of dendrotoxin-mediated neurotoxicity of black mamba venom by oligoclonal human IgG antibodies. Nat. Commun. 9, e3928. https://doi.org/10.1038/s41467-018-06086-4. Erratum in: Nat. Commun. 9, e4957.
Ledsgaard, L., Kilstrup, M., Karatt-Vellatt, A., McCafferty, J., Laustsen, A.H., 2018. Basics of antibody phage display technology. Toxins 10, e236. https://doi.org/10.3390/toxins10060236.
Lewin, M.R., Gilliam, L.L., Gilliam, J., Samuel, S.P., Bulfone, T.C., Bickler, P.E., Gutiérrez, J.M., 2018a. Delayed LY333013 (oral) and LY315920 (intravenous) reverse severe neurotoxicity and rescue juvenile pigs from lethal doses of Micrurus fulvius (eastern coral snake) venom. Toxins (Basel) 10, E479. https://doi.org/10.3390/toxins10110479.
Lewin, M.R., Gutiérrez, J.M., Samuel, S.P., Herrera, M., Bryan-Quirós, W., Lomonte, B., Bickler, P.E., Bulfone, T.C., Williams, D.J., 2018b. Delayed oral LY333013 rescues mice from highly neurotoxic, lethal doses of papuan taipan (oxyuranus scutellatus) venom. Toxins (Basel) 10, E380. https://doi.org/10.3390/toxins10100380.
Li, B., Tom, J.Y.K., Oare, D., Yen, R., Fairbrother, W.J., Wells, J.A., Cunningham, B.C., 1995. Minimization of a polypeptide hormone. Science 270, 1657–1660. https://doi.org/10.1126/science.270.5242.1657.
Lifton, R.P., Goldberg, M.L., Karp, R.W., Hogness, D.S., 1978. The organization of the histone genes in Drosophila melanogaster: functional and evolutionary implications. Cold Spring Harbor Symp. Quant. Biol. 42, 1047–1051.
Lincz, L.F., Lonergan, A., Scorgie, F.E., Rowlings, P., Gibson, R., Lawrie, A., Seldon, M., 2006. Endogenous thrombin potential for predicting risk of venous thromboembolism in carriers of factor V Leiden. Pathophysiol. Haemostasis Thrombosis 35, 435–439.
Lisy, O., Huntley, B.K., McCormick, D.J., Kurlansky, P.A., Burnett Jr., J.C., 2008. Design, synthesis, and actions of a novel chimeric natriuretic peptide: cd-np. J. Am. Coll. Cardiol. 52, 60–68. https://doi.org/10.1016/j.jacc.2008.02.077.
Lomonte, B., Escolano, J., Fernández, J., Sanz, L., Angulo, Y., Gutiérrez, J.M., Calvete, J. J., 2008. Snake venomics and antivenomics of the arboreal neotopical pitvipers Bothriechis lateralis and Bothriehis schlegelii. J. Proteome Res. 7, 2445–2457. https://doi.org/10.1021/pr8000139. Epub 2008 Apr 30.
Luna, M.S., Hortencio, T.M., Ferreira, Z.S., Yamanouye, N., 2009. Sympathetic outflow activates the venom gland of the snake Bothrops jararaca by regulating the activation of transcription factors and the synthesis of venom gland proteins. J. Exp. Biol. 212, 1535–1543. https://doi.org/10.1242/jeb.030197.
Lynch, V.J., 2007. Inventing an arsenal: adaptive evolution and neofunctionalization of snakevenom phospholipase A2 genes. BMC Evol. Biol. 7, 2.
Lynch, J.J., Cook, J.J., Sitko, G.R., Holahan, M.A., Ramjit, D.R., Mellott, M.J., Stranieri, M.T., Stabilito, I.I., Zhang, G., Lynch, R.J., 1995. Nonpeptide glycoprotein IIb/IIIa inhibitors. Antithrombotic effects of MK-0383. J. Pharmacol. Exp. Therapeut. 272, 20–32.
Lyukmanova, E.N., Shenkarev, Z.O., Shulepko, M.A., Mineev, K.S., D'Hoedt, D., Kasheverov, I.E., Filkin, S.Y., Krivolapova, A.P., Janickova, H., Dolezal, V., Dolgikh, D.A., Arseniev, A.S., Bertrand, D., Tsetlin, V.I., Kirpichnikov, M.P., 2011. NMR structure and action on nicotinic acetylcholine receptors of water-soluble domain of human LYNX1. J. Biol. Chem. 286, 10618–10627. https://doi.org/10.1074/jbc.M110.189100. Epub 2011 Jan 20.
Ma, D., Armugam, A., Jeyaseelan, K., 2001. Expression of cardiotoxin-2 gene. Cloning, characterization and deletion analysis of the promoter. Eur. J. Biochem. 268, 1844–1850.
Ma, D., Armugam, A., Jeyaseelan, K., 2002. Alpha-neurotoxin gene expression in Naja sputatrix: identification of a silencer element in the promoter region. Arch. Biochem. Biophys. 404, 98–105.
Maack, T., Atlas, S.A., Camargo, M.J., Cogan, M.G., 1986. Renal hemodynamic and natriuretic effects of atrial natriuretic factor. Fed. Proc. 45, 2128–2132.
Mahjoub, Y., Malaquin, S., Mourier, G., Lorne, E., Abou Arab, O., Massy, Z.A., Dupont, H., Ducancel, F., 2015. Short- versus long-sarafotoxins: two structurally related snake toxins with very different in vivo haemodynamic effects. PloS One 10, e0132864, 10.1371/journal.pone.0132864.eCollection 2015.
Marsh, N.A., 1998. Use of snake venom fractions in the coagulation laboratory. Blood Coagul. Fibrinolysis 9, 395–404.
Marsh, N.A., 2001. Diagnostic uses of snake venom. Haemostasis 31, 211–217.
Marsh, N., Williams, V., 2005. Practical applications of snake venom toxins in haemostasis. Toxicon 45, 1171–1181.
McCafferty, J., Griffiths, A.D., Winter, G., Chiswell, D.J., 1990. Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348, 552–554.
McCleary, R.J., Kini, R.M., 2013. Non-enzymatic proteins from snake venoms: a gold mine of pharmacological tools and drug leads. Toxicon 62, 56–74. https://doi.org/10.1016/j.toxicon.2012.09.008. Epub 2012 Oct 8.
Minh Le, T.N., Reza, M.A., Swarup, S., Kini, R.M., 2005. Gene duplication of coagulation factor V and origin of venom prothrombin activator in Pseudonaja textilis snake. Thromb. Haemostasis 93, 420–429.
Miwa, J.M., Ibanez-Tallon, I., Crabtree, G.W., Sánchez, R., Sali, A., Role, L.W., Heintz, N., 1999. lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. Neuron 23, 105–114.
Modahl, C.M., Brahma, R.K., Koh, C.Y., Shioi, N., Kini, R.M., 2020. Omics technologies for profiling toxin diversity and evolution in snake venom: impacts on the discovery of therapeutic and diagnostic agents. Annu. Rev. Anim. Biosci. 8, 91–116. https://doi.org/10.1146/annurev-animal-021419-083626. Epub 2019 Nov 8.
Momic, T., Katzhendler, J., Shai, E., Noy, E., Senderowitz, H., Eble, J.A., Marcinkiewicz, C., Varon, D., Lazarovici, P., 2015. Vipegitide: a folded peptidomimetic partial antagonist of α2β1 integrin with antiplatelet aggregation activity. Drug Des. Dev. Ther. 9, 291–304.
Morita, T., 2004. C-type lectin-related proteins from snake venoms. Curr. Drug Targets - Cardiovasc. Hematol. Disord. 4, 357–373.
Moriyama, Y., Koshiba-Takeuchi, K., 2018. Significance of whole-genome duplications on the emergence of evolutionary novelties. Brief Funct. Genomics 17, 329–338. https://doi.org/10.1093/bfgp/ely007.
Mourier, G., Hajj, M., Cordier, F., Zorba, A., Gao, X., Coskun, T., Herbet, A., Marcon, E., Beau, F., Delepierre, M., Ducancel, F., Servent, D., 2012. Pharmacological and structural characterization of long-sarafotoxins, a new family of endothelin-like peptides: role of the C-terminus extension. Biochimie 94, 461–470. https://doi.org/10.1016/j.biochi.2011.08.014. Epub 2011 Aug 29.
Mullard, A., 2019. FDA drug approvals. Nat. Rev. Drug Discov. 18, 85–89. https://doi.org/10.1038/d41573-019-00014-x.
Nakashima, K., Ogawa, T., Oda, N., Hattori, M., Sakaki, Y., Kihara, H., Ohno, M., 1993. Accelerated evolution of Trimeresurus flavoviridis venom gland phospholipase A2 isozymes. Proc. Natl. Acad. Sci. U.S.A. 90, 5964–5968.
Nakashima, K., Nobuhisa, I., Deshimaru, M., Nakai, M., Ogawa, T., Shimohigashi, Y., Fukumaki, Y., Hattori, M., Sakaki, Y., Hattori, S., Ohno, M., 1995. Accelerated evolution in the protein-coding regions is universal in crotalinae snake venom gland phospholipase A2 isozyme genes. Proc. Natl. Acad. Sci. U.S.A. 92, 5605–5609.
Naufer, M.N., Furano, A.V., Williams, M.C., 2019. Protein-nucleic acid interactions of LINE-1 ORF1p. Semin. Cell Dev. Biol. 86, 140–149. https://doi.org/10.1016/j.semcdb.2018.03.019. Epub 2018 Mar 31.
Newman, D.L., Thurgood, L.A., Gregory, S.L., 2019. The impact of aneuploidy on cellular homeostasis. Free Radic. Res. 53, 705–713. https://doi.org/10.1080/10715762.2019.1623398. Epub 2019 Jun 7.
Ng, A.S., Kini, R.M., 2013. Structural determinants in protein folding: a single conserved hydrophobic residue determines folding of EGF domains. ACS Chem. Biol. 8, 161–169. https://doi.org/10.1021/cb300445a. Epub 2012 Oct 24.
Nirthanan, S., Charpantier, E., Gopalakrishnakone, P., Gwee, M.C., Khoo, H.E., Cheah, L. S., Bertrand, D., Kini, R.M., 2002. Candoxin, a novel toxin from Bungarus candidus, is a reversible antagonist of muscle (alphabetagammadelta) but a poorly reversible antagonist of neuronal alpha 7 nicotinic acetylcholine receptors. J. Biol. Chem. 277, 17811–17820.
Nobuhisa, I., Nakashima, K., Deshimaru, M., Ogawa, T., Shimohigashi, Y., Fukumaki, Y., Sakaki, Y., Hattori, S., Kihara, H., Ohno, M., 1996. Accelerated evolution of Trimeresurus okinavensis venom gland phospholipase A2 isozyme-encoding genes. Gene 172, 267–272.
Nurden, P., Jandrot-Perrus, M., Combrié, R., Winckler, J., Arocas, V., Lecut, C., Pasquet, J.M., Kunicki, T.J., Nurden, A.T., 2004. Severe deficiency of glycoprotein VI in a patient with gray platelet syndrome. Blood 104, 107–114.
Ogawa, T., Oda, N., Nakashima, K., Sasaki, H., Hattori, M., Sakaki, Y., Kihara, H., Ohno, M., 1992. Unusually high conservation of untranslated sequences in cDNAs for Trimeresurus flavoviridis phospholipase A2 isozymes. Proc. Natl. Acad. Sci. U.S.A. 89, 8557–8561.
Ogawa, H., Qiu, Y., Ogata, C.M., Misono, K.S., 2004. Crystal structure of hormone-bound atrial natriuretic peptide receptor extracellular domain: rotation mechanism for transmembrane signal transduction. J. Biol. Chem. 279, 28625–28631. https://doi.org/10.1074/jbc.M313222200.
Ogawa, T., Chijiwa, T., Oda-Ueda, N., Ohno, M., 2005. Molecular diversity and accelerated evolution of C-type lectin-like proteins from snake venom. Toxicon 45, 1–14.
Ogawa, H., Qiu, Y., Huang, L., Tam-Chang, S.W., Young, H.S., Misono, K.S., 2009. Structure of the atrial natriuretic peptide receptor extracellular domain in the unbound and hormone-bound states by single-particle electron microscopy. FEBS J. 276, 1347–1355. https://doi.org/10.1111/j.1742-4658.2009.06870.x.
Ogbourne, S., Antalis, T.M., 1998. Transcriptional control and the role of silencers in transcriptional regulation in eukaryotes. Biochem. J. 331, 1–14.

Ohno, S., 1970. Evolution by Gene Duplication. Springer, ISBN 978-3-642-86661-6, pp. 1–160.
Ohno, M, Ménez, R, Ogawa, T, Danse, JM, Shimohigashi, Y, Fromen, C, Ducancel, F, Zinn-Justin, S, Le Du, MH, Boulain, JC, Tamiya, T, Ménez, A, 1998. Molecular evolution of snake toxins: is the functional diversity of snake toxins associated with a mechanism of accelerated evolution? Prog. Nucl. Acid Res. Mol. Biol. 59, 307–364.
Olins, G.M., Patton, D.R., Bovy, P.R., Mehta, P.P., 1988. A linear analog of atrial natriuretic peptide (ANP) discriminates guanylate cyclase-coupled ANP receptors from non-coupled receptors. J. Biol. Chem. 263, 10989–10993. PMID:2899082.
Oron, U., Bdolah, A., 1973. Regulation of protein synthesis in the venom gland of viperid snakes. J. Cell Biol. 56, 177–190.
Oron, U., Bdolah, A., 1978. Intracellular transport of proteins in active and resting secretory cells of the venom gland of Vipera palaestinae. J. Cell Biol. 78, 488–502.
Pachiappan, A., Thwin, M.M., Manikandan, J., Gopalakrishnakone, P., 2005. Glial inflammation and neurodegeneration induced by candoxin, a novel neurotoxin from Bungarus candidus venom: global gene expression analysis using microarray. Toxicon 46, 883–899.
Paine, M.J.I., Desmond, H.P., Theakston, R.D.G., Crampton, J.M., 1992. Purifcation, cloning, and molecular characterization of a high molecular weight hemorrhagic metalloproteinase, jararhagin, from Bothrops jararaca venom. Insights into the disintegrin gene family. J. Biol. Chem. 267, 22869–22876.
Paine, M.J.I., Moura-De-Silva, A.M., Theakston, R.D.G., Crampton, J.M., 1994. Cloning of metalloproteinase genes in the carpet viper (Echis pyramidum leakeyi). Further members of the metallo- protease/disintegrin gene family. Eur. J. Biochem. 224, 483–488.
Parmley, S.F., Smith, G.P., 1988. Antibody-selectable filamentous fd phage vectors: affinity purification of target genes. Gene 73, 305–318.
Perchuc, A.M., Wilmer, M., 2010. Diagnostic use of snake venom components in the coagulation laboratory. In: Kini, R.M., Clemetson, K.J., Markland, F.S., McLane, M. A., Morita, T. (Eds.), Toxins and Hemostasis: from Bench to Bedside. Springer, Dordrecht, pp. 747–766, 2010.
Perry, B.W., Card, D.C., McGlothlin, J.W., Pasquesi, G.I.M., Adams, R.H., Schield, D.R., Hales, N.R., Corbin, A.B., Demuth, J.P., Hoffmann, F.G., Vandewege, M.W., Schott, R.K., Bhattacharyya, N., Chang, B.S.W., Casewell, N.R., Whiteley, G., Reyes-Velasco, J., Mackessy, S.P., Gamble, T., Storey, K.B., Biggar, K.K., Passow, C.N., Kuo, C.H., McGaugh, S.E., Bronikowski, A.M., de Koning, A.P.J., Edwards, S.V., Pfrender, M.E., Minx, P., Brodie 3rd, E.D., Brodie Jr., E.D., Warren, W.C., Castoe, T. A., 2018. Molecular adaptations for sensing and securing prey and insight into amniote genome diversity from the garter snake genome. Genome Biol. Evol. 10, 2110–2129. https://doi.org/10.1093/gbe/evy157.
Phillips, D.R., Scarborough, R.M., 1997. Clinical pharmacology of eptifibatide. Am. J. Cardiol. 80, 11B–20B.
Pollock, J., Coffman, J., Ho, S.V., Farid, S.S., 2017. Integrated continuous bioprocessing: economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. Biotechnol. Prog. 33, 854–866. https://doi.org/10.1002/btpr.2492. Epub 2017 Jun 2.
Post, Y., Puschhof, J., Beumer, J., Kerkkamp, H.M., de Bakker, M.A.G., Slagboom, J., de Barbanson, B., Wevers, N.R., Spijkers, X.M., Olivier, T., Kazandjian, T.D., Ainsworth, S., Iglesias, C.L., van de Wetering, W.J., Heinz, M.C., van Ineveld, R.L., van Kleef, R.G.D.M., Begthel, H., Korving, J., Bar-Ephraim, Y.E., Getreuer, W., Rios, A.C., Westerink, R.H.S., Snippert, H.J.G., van Oudenaarden, A., Peters, P.J., Vonk, F.J., Kool, J., Richardson, M.K., Casewell, N.R., Clevers, H., 2020. Snake Venom Gland Organoids. Cell 180, 233–247. https://doi.org/10.1016/j.cell.2019.11.038.
Prado-Franceschi, J., Brazil, O.V., 1981. Convulxin, a new toxin from the venom of the South American rattlesnake Crotalus durissus terrificus. Toxicon 19, 875–887.
Rademaker, M.T., Scott, N.J.A., Koh, C.Y., Kini, R.M., Richards, A.M., 2020. Natriuretic peptide analogues with distinct vasodilatory or renal activity: integrated effects in health and experimental heart failure. Cardiovasc. Res. Mar 13. https://doi.org/10.1093/cvr/cvaa052 pii: cvaa052, ([Epub ahead of print]).
Rao, V.S., Kini, R.M., 2002. Pseutarin C, a prothrombin activator from Pseudonaja textilis venom: its structural and functional similarity to mammalian coagulation factor Xa-Va complex. Thromb. Haemostasis 88, 611–619.
Rao, V.S., Joseph, J.S., Kini, R.M., 2003a. Group D prothrombin activators from snake venom are structural homologues of mammalian blood coagulation factor Xa. Biochem. J. 369, 635–642.
Rao, V.S., Swarup, S., Kini, R.M., 2003b. The nonenzymatic subunit of pseutarin C, a prothrombin activator from eastern brown snake (Pseudonaja textilis) venom, shows structural similarity to mammalian coagulation factor V. Blood 102, 1347–1354.
Rawat, S., Laing, G., Smith, D.C., Theakston, D., Landon, J., 1994. A new antivenom to treat eastern coral snake (Micrurus fulvius fulvius) envenoming. Toxicon 32, 185–190.
Read, M.S., Shermer, R.W., Brinkhous, K.M., 1978. Venom coagglutinin – activator of platelet-aggregation dependent on von Willebrand factor. Proc. Nat. Acad. Sci., USA 75, 4514–4518.
Rees, D.C., Williams, T.N., Gladwin, M.T., 2010. Sickle-cell disease. Lancet 376, 2018–2031. https://doi.org/10.1016/S0140-6736(10)61029-X. Epub 2010 Dec 3.
Reza, M.A., Swarup, S., Kini, R.M., 2005. Gene structures of trocarin D and coagulation factor X, two functionally diverse prothrombin activators from Australian rough scaled snake. Pathophysiol. Haemostasis Thrombosis 34, 205–208.
Reza, M.A., Minh Le, T.N., Swarup, S., Kini, R.M., 2006. Molecular evolution caught in action: gene duplication and evolution of molecular isoforms of prothrombin activators in Pseudonaja textilis (brown snake). J. Thromb. Haemostasis 4, 1346–1353.
Reza, M.A., Swarup, S., Kini, R.M., 2007. Structure of two genes encoding parallel prothrombin activators in Tropidechis carinatus snake: gene duplication and recruitment of factor X gene to the venom gland. J. Thromb. Haemostasis 5, 117–126.
Rodi, D.J., Makowski, L., 1999. Phage-display technology—finding a needle in a vast molecular haystack. Curr. Opin. Biotechnol. 10, 87–93.
Roncolato, E.C., Campos, L.B., Pessenda, G., Costa e Silva, L., Furtado, G.P., Barbosa, J. E., 2015. Phage display as a novel promising antivenom therapy: a review. Toxicon 93, 79–84. https://doi.org/10.1016/j.toxicon.2014.11.001. Epub 2014 Nov 5.
Rooney, A.M., McNally, T., Mackie, I.J., Machin, S.J., 1994. The Taipan snake venom time: a new test for lupus anticoagulant. J. Clin. Pathol. 47, 497–501.
Rotenberg, D., Bamberger, E.S., Kochva, E., 1971. Studies on ribonucleic acid synthesis in the venom glands of Vipera palaestinae (Ophidia, Reptilia). Biochem. J. 121, 609–612.
Salvador, G.H.M., Gomes, A.A.S., Bryan-Quirós, W., Fernández, J., Lewin, M.R., Gutiérrez, J.M., Lomonte, B., Fontes, M.R.M., 2019. Structural basis for phospholipase A2-like toxin inhibition by the synthetic compound Varespladib (LY315920). Sci. Rep. 9, e17203 https://doi.org/10.1038/s41598-019-53755-5.
Sanz, L., Quesada-Bernat, S., Chen, P.Y., Lee, C.D., Chiang, J.R., Calvete, J.J., 2018. Translational venomics: third-generation antivenomics of anti-siamese russell's viper, Daboia siamensis, antivenom manufactured in taiwan CDC's vaccine center. Trav. Med. Infect. Dis. 3, E66. https://doi.org/10.3390/tropicalmed3020066.
Sasaki, A., Kida, O., Kangawa, K., Matsuo, H., Tanaka, K., 1985. Hemodynamic effects of α-human atrial natriuretic polypeptide (α-hANP) in rats. Eur. J. Pharmacol. 109, 405–407. https://doi.org/10.1016/0014-2999(85)90403-0.
Sayinalp, N., Haznedaroğlu, I.C., Aksu, S., Büyükaşik, Y., Göker, H., Parlak, H., Ozcebe, O.I., Kirazli, S., Dündar, S.V., Gürgey, A., 2004. The predictability of factor V Leiden (FV:Q(506)) gene mutation via clotting-based diagnosis of activated protein C resistance. Clin. Appl. Thromb. Hemost. 10, 265–270.
Scarborough, R.M., 1999. Development of eptifibatide. Am. Heart J. 138, 1093–1104.
Scarborough, R.M., Naughton, M.A., Teng, W., Rose, J.W., Phillips, D.R., Nannizzi, L., Arfsten, A., Campbell, A.M., Charo, I.F., 1993. Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. J. Biol. Chem. 268, 1066–1073.
Schield, D.R., Card, D.C., Hales, N.R., Perry, B.W., Pasquesi, G.M., Blackmon, H., Adams, R.H., Corbin, A.B., Smith, C.F., Ramesh, B., Demuth, J.P., Betrán, E., Tollis, M., Meik, J.M., Mackessy, S.P., Castoe, T.A., 2019. The origins and evolution of chromosomes, dosage compensation, and mechanisms underlying venom regulation in snakes. Genome Res. 29, 590–601. https://doi.org/10.1101/gr.240952.118. Epub 2019 Mar 21.
Schweitz, H., Vigne, P., Moinier, D., Frelin, C., Lazdunski, M., 1992 Jul 15. A new member of the natriuretic peptide family is present in the venom of the green mamba (Dendroaspis angusticeps). J. Biol. Chem. 267 (20), 13928–13932.
Schoni, R., 2005. The use of snake venom-derived compounds for new functional diagnostic test kits in the field of haemostasis. Pathophysiol. Haemostasis Thrombosis 34, 234–240.
Segura, Á., Herrera, M., Villalta, M., Vargas, M., Gutiérrez, J.M., León, G., 2013. Assessment of snake antivenom purity by comparing physicochemical and immunochemical methods. Biologicals 41, 93–97. https://doi.org/10.1016/j.biologicals.2012.11.001. Epub 2012 Nov 26.
Seilhamer, J.J., Pruzanski, W., Vadas, P., Plant, S., Miller, J.A., Kloss, J., Johnson, L.K., 1989. Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. J. Biol. Chem. 264, 5335–5338.
Sells, P.G., Hommel, M., Theakston, R.D.G., 1989. Venom production in snakes venom gland cells cultured in vitro. Toxicon 27, 1245–1249.
Serjeant, G.R., 2010. One hundred years of sickle cell disease. Br. J. Haematol. 151, 425–429. https://doi.org/10.1111/j.1365-2141.2010.08419.x. Epub 2010 Oct 19.
Shibata, H., Chijiwa, T., Oda-Ueda, N., Nakamura, H., Yamaguchi, K., Hattori, S., Matsubara, K., Matsuda, Y., Yamashita, A., Isomoto, A., Mori, K., Tashiro, K., Kuhara, S., Yamasaki, S., Fujie, M., Goto, H., Koyanagi, R., Takeuchi, T., Fukumaki, Y., Ohno, M., Shoguchi, E., Hisata, K., Satoh, N., Ogawa, T., 2018. The habu genome reveals accelerated evolution of venom protein genes. Sci. Rep. 8, e11300 https://doi.org/10.1038/s41598-018-28749-4.
Siang, A.S., Doley, R., Vonk, F.J., Kini, R.M., 2010. Transcriptomic analysis of the venom gland of the red-headed krait (Bungarus flaviceps) using expressed sequence tags. BMC Mol. Biol. 11, e24. https://doi.org/10.1186/1471-2199-11-24.
Sidhu, S.S., 2000. Phage display in pharmaceutical biotechnology. Curr. Opin. Biotechnol. 11, 610–616.
Skolovsky, M., Galron, R., Kloog, Y., Bdolah, A., Indig, F.E., Blumberg, S., Fleminger, G., 1990. Endothelins are more sensitive than sarafotoxins to neutral endopeptidase: possible physiological significance. Proc. Natl. Acad. Sci. U.S.A. 87, 4702–4706.
Sridharan, S., Kini, R.M., 2015. Tail wags the dog: activity of krait natriuretic peptide is determined by its C-terminal tail in a natriuretic peptide receptor-independent manner. Biochem. J. 469, 255–266. https://doi.org/10.1042/BJ20150281.
Sridharan, S., Kini, R.M., 2018. Decoding the molecular switches of natriuretic peptides which differentiate its vascular and renal functions. Biochem. J. 475, 399–413. https://doi.org/10.1042/BCJ20170690.
Sridharan, S., Kini, R.M., Richards, A.M., 2020. Venom natriuretic peptides guide the design of heart failure therapeutics. Pharmacol. Res. 155, 104687. https://doi.org/10.1016/j.phrs.2020.104687 ([Epub ahead of print]).
Srinivasan, K.N., Nirthanan, S., Sasaki, T., Sato, K., Cheng, B., Gwee, M.C., Kini, R.M., Gopalakrishnakone, P., 2001. Functional site of bukatoxin, an alpha-type sodium channel neurotoxin from the Chinese scorpion (Buthus martensi Karsch) venom: probable role of the (52)PDKVP(56) loop. FEBS Lett. 494, 145–149.
Strydom, D.J., 1973a. Protease inhibitors as snake venom toxins. Nat. New Biol. 243, 88–89.
Strydom, D.J., 1973b. Snake venom toxins. Structure-function relationships and phylogenetics. Comp. Biochem. Physiol. B 44, 269–281.

Suga, S., Nakao, K., Hosoda, K., Mukoyama, M., Ogawa, Y., Shirakami, G., Arai, H., Saito, Y., Kambayashi, Y., Inouye, K., 1992. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. Endocrinology 130, 229–239. https://doi.org/10.1210/endo.130.1.1309330.

Suga, S., Itoh, H., Komatsu, Y., Ogawa, Y., Hama, N., Yoshimasa, T., Nakao, K., 1993. Cytokine-induced C-type natriuretic peptide (CNP) secretion from vascular endothelial cells – evidence for CNP as a novel autocrine/paracrine regulator from endothelial cells. Endocrinology 133, 3038–3041. https://doi.org/10.1210/endo.133.6.8243333.

Sunagar, K., Jackson, T.N., Undheim, E.A., Ali, S.A., Antunes, A., Fry, B.G., 2013. Three-fingered RAVERs: rapid accumulation of variations in exposed residues of snake venom toxins. Toxins 5, 2172–2208. https://doi.org/10.3390/toxins5112172.

Suryamohan, K., Krishnankutty, S.P., Guillory, J., Jevit, M., Schröder, M.S., Wu, M., Kuriakose, B., Mathew, O.K., Perumal, R.C., Koludarov, I., Goldstein, L.D., Senger, K., Dixon, M.D., Velayutham, D., Vargas, D., Chaudhuri, S., Muraleedharan, M., Goel, R., Chen, Y.J., Ratan, A., Liu, P., Faherty, B., de la Rosa, G., Shibata, H., Baca, M., Sagolla, M., Ziai, J., Wright, G.A., Vucic, D., Mohan, S., Antony, A., Stinson, J., Kirkpatrick, D.S., Hannoush, R.N., Durinck, S., Modrusan, Z., Stawiski, E.W., Wiley, K., Raudsepp, T., Kini, R.M., Zachariah, A., Seshagiri, S., 2020. The Indian cobra reference genome and transcriptome enables comprehensive identification of venom toxins. Nat. Genet. 52, 106–117. https://doi.org/10.1038/s41588-019-0559-8. Epub 2020 Jan 6.

Takayanagi, R., Kitazumi, K., Takasaki, C., Ohnaka, K., Aimoto, S., Tasaka, K., Ohashi, M., Nawata, H., 1991. Presence of non-selective type of endothelin receptor on vascular endothelium and its linkage to vasodilation. FEBS Lett. 282, 103–106.

Takeya, H., Oda, K., Miyata, T., Omori-Satoh, T., Iwanaga, S., 1990. The complete amino acid sequence of the high molecular mass hemorrhagic protein HRIB isolated from the venom of Trimeresurus flavoviridis. J. Biol. Chem. 265, 16068–16073.

Tamiya, T., Fujimi, T.J., 2006 Nov. Molecular evolution of toxin genes in Elapidae snakes. Mol. Divers. 10 (4), 529–543.

Tan, N.H., 1983. Improvement of Malayan cobra (Naja naja sputatrix) antivenin. Toxicon 21, 75–79.

Tan, D.C., Kini, R.M., Jois, S.D., Lim, D.K., Xin, L., Ge, R., 2001. A small peptide derived from Flt-1 (VEGFR-1) functions as an angiogenic inhibitor. FEBS Lett. 494, 150–156.

Tasoulis, T., Isbister, G.K., 2017. A review and database of snake venom proteomes. Toxins 9, E290. https://doi.org/10.3390/toxins9090290.

Theakston, R.D.G., Warrell, D.A., Griffiths, E., 2003. Report of a WHO workshop on the standardization and control of antivenoms. Toxicon 41, 541–557.

Thiagarajan, P., Pengo, V., Shapiro, S.S., 1986. The use of the dilute Russell viper venom time for the diagnosis of lupus anticoagulants. Blood 68, 869–874.

Triplett, D.A., Stocker, K.F., Unger, G.A., Barna, L.K., 1993. The Textarin/Ecarin ratio: a confirmatory test for lupus anticoagulants. Thromb. Haemostasis 70, 925–931.

Utkin, Y.N., Kuch, U., Kasheverov, I.E., Lebedev, D.S., Cederlund, E., Molles, B.E., Polyak, I., Ivanov, I.A., Prokopev, N.A., Ziganshin, R.H., Jornvall, H., Alvelius, G., Chanhome, L., Warrell, D.A., Mebs, D., Bergman, T., Tsetlin, V.I., 2019. Novel long-chain neurotoxins from Bungarus candidus distinguish the two binding sites in muscle-type nicotinic acetylcholine receptors. Biochem. J. 476, 1285–1302. https://doi.org/10.1042/BCJ20180909.

van Bladel, E.R., Laarhoven, A.G., van der Heijden, L.B., Heitink-Pollé, K.M., Porcelijn, L., van der Schoot, C.E., de Haas, M., Roest, M., Vidarsson, G., de Groot, P. G., Bruin, M.C., 2014. Functional platelet defects in children with severe chronic ITP as tested with 2 novel assays applicable for low platelet counts. Blood 123, 1556–1563. https://doi.org/10.1182/blood-2013-08-519686.

Venugopal, S., Kao, C., Chandna, R., Sulochana, K.N., Subramanian, V., Chen, M., Kini, R. M., Ge, R., 2018. Angio-3, a 10-residue peptide derived from human plasminogen kringle 3, suppresses tumor growth in mice via impeding both angiogenesis and vascular permeability. Angiogenesis 21, 653–665. https://doi.org/10.1007/s10456-018-9616-7. Epub 2018 Apr 24.

Viana, L.G., Valente, R.H., Heluany, C.S., Souza-Imberg, A., Luna, M.S., Perales, J., Yamanouye, N., 2017. Bothrops jararaca venom gland secretory cells in culture: effects of noradrenaline on toxin production and secretion. Toxicon 133, 1–9. https://doi.org/10.1016/j.toxicon.2017.04.009.

Viguera, E., Canceill, D., Ehrlich, S.D., 2001. Replication slippage involves DNA polymerase pausing and dissociation. EMBO J. 20, 2587–2595.

Vogel, C.W., Smith, C.A., Müller-Eberhard, H.J., 1984. Cobra venom factor: structural homology with the third component of human complement. J. Immunol. 133, 3235–3241.

Waiddyanatha, S., Silva, A., Siribaddana, S., Isbister, G.K., 2019. Long-term effects of snake envenoming. Toxins 11, E193. https://doi.org/10.3390/toxins11040193.

Waldman, S.A., Rapoport, R.M., Murad, F., 1984. Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. J. Biol. Chem. 259, 14332–14334.

Walsh, G., 2014. Biopharmaceutical benchmarks 2014. Nat. Biotechnol. 32, 992–1000. https://doi.org/10.1038/nbt.3040.

Walsh, G., 2018. Biopharmaceutical benchmarks 2018. Nat. Biotechnol. 36, 1136–1145. https://doi.org/10.1038/nbt.4305.

Weiser, E., Wollberg, Z., Kochva, E., Lee, S.Y., 1984. Cardiotoxic effects of the venom of the burrowing asp, Atractaspis engaddensis (Atractaspididae, Ophidia). Toxicon 22, 767–774.

Williams Jr., D.L., Jones, K.L., Pettibone, D.J., Lis, E.V., Clineschmidt, B.V., 1991. Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. Biochem. Biophys. Res. Commun. 175, 556–561.

Williams, D.J., Faiz, M.A., Abela-Ridder, B., Ainsworth, S., Bulfone, T.C., Nickerson, A. D., Habib, A.G., Junghanss, T., Fan, H.W., Turner, M., Harrison, R.A., Warrell, D.A., 2019. Strategy for a globally coordinated response to a priority neglected tropical disease: snakebite envenoming. PLoS Neglected Trop. Dis. 13, e0007059 https://doi.org/10.1371/journal.pntd.0007059.

Wisner, A., Braud, S., Bon, C., 2001. Snake venom proteinases as tools in hemostasis studies: structure-function relationship of a plasminogen activator purified from Trimeresurus stejnegeri venom. Haemostasis 31, 133–140.

Wollberg, Z., Shabo-Shina, R., Intrator, N., Bdolah, A., Kochva, E., Shavit, G., Oron, Y., Vidne, B.A., Gitter, S., 1988. A novel cardiotoxic polypeptide from the venom of Atractaspis engaddensis (burrowing asp): cardiac effects in mice and isolated rat and human heart preparations. Toxicon 26, 525–534.

Yamanouye, N., Kerchove, C.M., Moura-da-Silva, A.M., Carneiro, S.M., Markus, R.P., 2004. Long-term primary culture of secretory cells of Bothrops jararaca venom gland for venom production in vitro. Nat. Protoc. 1, 2763–2766.

Yamazaki, Y., Takani, K., Atoda, H., Morita, T., 2003. Snake venom vascular endothelial growth factors (VEGFs) exhibit potent activity through their specific recognition of KDR (VEGF receptor 2). J. Biol. Chem. 278, 51985–51988.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.

Yang, C., Bolotin, E., Jiang, T., Sladek, F.M., Martinez, E., 2007. Prevalence of the initiator over the TATA box in human and yeast genes and identification of DNA motifs enriched in human TATA-less core promoters. Gene 389, 52–65.

Yang, O., Qadan, M., Ierapetritou, M., 2019. Economic analysis of batch and continuous biopharmaceutical antibody production: a review. J. Pharm. Innov. 14, 1–19. https://doi.org/10.1007/s12247-018-09370-4. Epub 2019 Jan 25.

Yawn, B.P., Buchanan, G.R., Afenyi-Annan, A.N., Ballas, S.K., Hassell, K.L., James, A.H., Jordan, L., Lanzkron, S.M., Lottenberg, R., Savage, W.J., Tanabe, P.J., Ware, R.E., Murad, M.H., Goldsmith, J.C., Ortiz, E., Fulwood, R., Horton, A., John-Sowah, J., 2014. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. J. Am. Med. Assoc. 312, 1033–1048. https://doi.org/10.1001/jama.2014.10517.

Yin, W., Wang, Z.J., Li, Q.Y., Lian, J.M., Zhou, Y., Lu, B.Z., Jin, L.J., Qiu, P.X., Zhang, P., Zhu, W.B., Wen, B., Huang, Y.J., Lin, Z.L., Qiu, B.T., Su, X.W., Yang, H.M., Zhang, G. J., Yan, G.M., Zhou, Q., 2016. Evolutionary trajectories of snake genes and genomes revealed by comparative analyses of five-pacer viper. Nat. Commun. 7, e13107 https://doi.org/10.1038/ncomms13107.

Zakeri, R., Burnett Jr., J.C., 2011. Designer natriuretic peptides: a vision for the future of heart failure therapeutics. Can. J. Physiol. Pharmacol. 89, 593–601. https://doi.org/10.1139/y11-048.

Zhan, C., Li, B., Hu, L., Wei, X., Feng, L., Fu, W., Lu, W., 2011. Micelle-based brain-targeted drug delivery enabled by a nicotine acetylcholine receptor ligand. Angew Chem. Int. Ed. Engl. 50, 5482–5485. https://doi.org/10.1002/anie.201100875.